A TEXTBOOK OF
VERTEBRATE EMBRYOLOGY

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VERTEBRATE EMBRYOLOGY

BY

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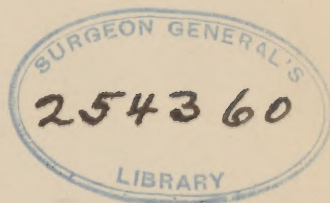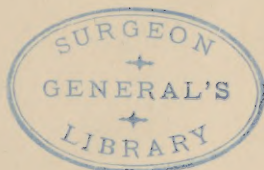
[1923]

To
My Mother

ERRATUM

P. 82, line 16, omit *the object of*

PREFACE

THIS book is designed as an introductory text in Vertebrate Embryology, a work which seems to be justified on the following grounds: The older texts upon this subject, though in many cases excellent, do not cover exactly the field which is now covered in many colleges; these texts, moreover, are becoming somewhat out of date in various details. Among the newer books the best ones tend to do one of two things. Either, in the interest of thoroughness, they confine their attention entirely to one form; e.g., the Chick, or else, for the sake of a broader viewpoint, they deal with a considerable number of animals, but in doing so touch only upon the earlier developmental stages of each. Now it is obvious that there is great value for the student, both in the accuracy gained by the careful intensive study of a single type, and also in the possession of less detailed knowledge of the history of other forms which are nearly related to it. Hence, what has seemed to be needed was a book which would, so far as is possible, make available both these advantages. To meet this need, the major part of the present text comprises a moderately complete account of the development of two typical forms; i.e., the Frog and the Chick, each of which, in the writer's opinion, has special features which justify such treatment. These relatively detailed discussions are then supplemented by chapters which present brief comparisons, not only with the Mammal, but also with certain other significant members of the Vertebrate group. Furthermore, the essentially embryological portion of the book is preceded by an optional introductory chapter dealing with the elements of cytology. Upon this basis the effort throughout the work has been to produce something especially adapted to the requirements of the general student of Zoölogy, as well as to the individual particularly interested in premedical preparation.

As regards certain details concerning the method of handling the topics involved, the following remains to be said. Because of the character of the book the chapter upon cytology places special emphasis upon the structure, development, and function of the germ cells, with particular reference to nuclear phenomena and their genetic sig-

v

nificance. The strictly embryological subject matter is then introduced
by a short general discussion of the more fundamental and universal
processes of Vertebrate development from the comparative standpoint.
This includes a description of the various types of segmentation, gas-
trulation, and the formation of the rudiments of the nervous system
and the main mesodermal structures. Following these introductory
chapters, Amphioxus is the first particular type to be considered be-
cause of the relatively primitive character of most of its early history.
The later development of this animal; i.e., that following the forma-
tion of the mesodermal somites, is, however, quite highly specialized
in respects which distinguish it from the vast majority of Chordates.
As these later stages are without great significance for the general
student, they are omitted.

The Frog, as suggested above, is one of the two forms which have
been treated at some length. The reasons for such extended considera-
tion in this instance and in that of the Chick are presumably obvious
to every Zoölogist. For the sake of the student, however, the value
of these animals as subjects of embryological study are indicated in
the paragraphs of the text which introduce them. In the case of the
Frog, its early history has been presented under the head of certain
fairly well recognized stages which lend themselves well to corre-
lation with work in the laboratory. In further pursuance of this method
the internal changes have been noted in alternation with those
occurring externally. This was done in order that the reader might
obtain, so far as possible, a correct idea of the really simultaneous
character of these processes. It did not seem feasible, however, in a
work of this scope to continue this plan throughout the entire course
of development in this animal. The later external changes, therefore,
are included under one heading, while the more advanced details of
organogeny are described in terms of particular systems.

Following the treatment of the Frog, there has been introduced a
very brief account of segmentation and gastrulation in the Teleosts and
the Gymnophiona. This has been done despite the realization that
in the case of the latter group laboratory consideration will in most
cases be impossible. The reason for this is the author's opinion that
segmentation and gastrulation in these two classes of animals are ex-
tremely valuable in assisting the student to relate these processes in
the Frog to those which he is about to study in the Bird. Experience,
moreover, has seemed to indicate that the relation of avian and mam-

malian gastrulation, to that in more primitive forms is always particu-
larly difficult for the beginner to grasp, and it is believed, therefore,
that any legitimate aid to this end is worth while.

In treating the early stages of the Chick a good deal of stress has been
placed upon the method of segmentation and gastrulation. The latter
especially has been emphasized because of its peculiar character, and
the desirability of making clear its relationship to that in the forms
already studied. The later history of this animal is then presented
in daily periods, according to the well known plan of Foster and
Balfour. This has been done because it seems to the writer that at
least in a beginning course this method has certain marked advantages
over that of studying the complete embryology of one system at a
time. In the first place the Bird lends itself particularly well to treat-
ment by periods, and secondly the simultaneous development of all
the systems is what is actually seen to occur in any animal. This
latter fact it would seem well to impress upon the student when pos-
sible by the method of presentation. Finally it has appeared not only
possible but easier to conduct the class work in correlation with the
laboratory when development is studied by periods rather than by
systems. It should be noted, nevertheless, that in this book the
material has been so arranged that the student can readily follow
through the complete growth of any one system if the instructor so
desires.

As regards the Mammals, it is felt that the detailed differences be-
tween the organogeny of this group and that of the Birds, are not, on
the whole, of great general biological significance. Of very consider-
able significance, however, are those unique characteristics of both
mother and embryo connected with mammalian gestation. For this
reason the discussion in this portion of the text is confined chiefly to
the earlier developmental stages, which are treated largely from the
comparative standpoint. The subject is introduced by a description
of the structure and functions of the adult reproductive organs in the
same manner as in the case of preceding forms. This involves the
process of ovulation, and in that connection it has seemed worth while
to describe briefly the peculiar cyclic phenomena which accompany
this process in the mammalian female. Following this the comparative
idea is pursued with particular reference to the development of the ex-
tra-embryonic appendages. This is believed to be especially important
from an evolutionary viewpoint because it shows how these appendages,

already observed in the Chick, have been modified in the various Mammals. This discussion is naturally accompanied by a description of the structure and probable evolution of the placenta. For the general plan of treatment of these latter topics the author frankly acknowledges his indebtedness to Professor Jenkinson's excellent book, *Vertebrate Embryology*.

Concerning bibliographical material, references to the more important literature of each subject are appended to the chapter which concludes consideration of the topic in question. As intimated, it will be quite obvious that these references make no pretense of being exhaustive. Their object is rather merely to point the way to further study for the reader who desires it. This is done, first, because the present volume is intended primarily as a text rather than as a book of reference, and, secondly, because it is felt that the beginner's interest may be more effectively aroused in this manner than by presenting to him at once every reference available. The latter, if desired, can be readily obtained in the more advanced books which are cited.

It is recognized that illustrations constitute an extremely important feature in a text of this character, and the writer has spared no pains in the attempt to make the figures adequate both in number and quality. It will be evident, however, that the majority of them are not original. This is due to the fact that through the kindness of the authors and publishers indicated below, there were made available a large number of excellent illustrations, which it seemed hardly worth while to attempt to improve upon. Nevertheless, in every instance where it was felt that such improvement was possible, or where it appeared that a new figure would be profitable, original drawings have been inserted. Lastly, it remains to be stated in this connection that in the case of all borrowed illustrations, great care has been taken to have the illustration and the terms used in its legend agree with the respective description and terminology in the text. The desirability of this, especially in an elementary book, is obvious; yet, according to the writer's observation, it is a feature which is too frequently overlooked.

In conclusion I desire to express my appreciation of the following favors. To Professor Frank R. Lillie and to Henry Holt and Co., I am indebted for their generous permission to use a large number of figures from Lillie's *Development of the Chick;* to Professor T. H. Morgan, his co-authors, and Henry Holt and Co., for certain illustra-

tions from *The Mechanism of Mendelian Heredity;* to Henry Holt and
Co., for numerous figures from Kellicott's *General Embryology* and
Chordate Development; and to the Delegates and Secretary of the
Clarendon Press for a like favor as regards Jenkinson's *Vertebrate
Embryology.* It is also a pleasure to acknowledge a similar debt to
Professor Morgan and The Columbia University Press for figures
from *Heredity and Sex;* to Professor J. Playfair McMurrich and
P. Blakiston's Son and Co. for clichés from McMurrich's *Develop-
ment of the Human Body;* to P. Blakiston's Son and Co. for further
clichés from Minot's *Laboratory Text Book of Embryology;* to Messrs.
Longmans Green and Co. for clichés from *Quain's Anatomy;* to
Messrs. G. P. Putnam and Co., for permission to use again certain
figures from Marshall's *Vertebrate Embryology,* copied and slightly
modified by Kellicott; and to Professor O. Van der Stricht and Dr.
T. W. Todd for allowing the use of photomicrographs made in the
Anatomical Department of Western Reserve University Medical School
from preparations presented to that department by Professor Van der
Stricht. In all cases the illustrations thus borrowed are acknowledged
in the legends of the figures concerned.

I wish further to express particular gratitude to Professor T. H.
Morgan for reading and criticising the first half of the manuscript; to
Professor J. H. McGregor for performing a similar service for the en-
tire book; to Professor M. M. Metcalf for suggestions regarding the
earlier chapters; to my wife for assistance with the proof; and to
Professor R. G. Harrison for the identification of the frog larvae used
in making certain of my original drawings. Especial gratitude is also
felt for the constant interest and helpfulness shown by my colleagues,
Professors R. A. Budington and C. G. Rogers.

.   R. S. McE.

Oberlin College,
August 15, 1923.

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PART I

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CHAPTER I

THE CELL AND ITS FUNCTION IN REPRODUCTION

All organisms are made up of more or less specialized cells and their products, which had as their ultimate origin either a single germ cell, or more usually two fused germ cells; i.e., a fertilized egg or *zygote*. The adult animal or plant has then arisen chiefly through the processes of cell division, differentiation, and rearrangement. Furthermore, these processes are always ordered in such a way as to result finally in the production of an individual similar in its general characteristics to the parents from which the fertilized ovum was derived. Such processes applied to any given case, therefore, constitute individual development or *ontogeny,* and their study is termed embryology. Since, as has just been indicated, however, the processes in question depend chiefly or wholly upon the activities of cells, it is desirable to begin embryology by giving some attention to the character and behavior of these organic units.

It will be found convenient to describe first a typical or generalized cell, and to proceed from that to a discussion of the more specialized male and female reproductive cells. It seems best to divide the description of the typical cell into two main phases; i.e., that of rest and that of division. In this connection, however, it should be clearly understood that by a " resting " cell is meant merely one that is not dividing. Such a cell, nevertheless, is not really resting, but is undergoing all the metabolic changes which are a constant accompaniment of life. The appearance of rest results simply from the fact that these chemical changes are less obvious than the very evident act of division, and the terminology is therefore only one of descriptive convenience.

THE TYPICAL CELL

The Resting Phase

According to the definition of Leydig and Schultze, given over forty years ago, a cell is " a mass of protoplasm containing a nucleus " (Fig. 1). This definition is applicable to the majority of cells, but in
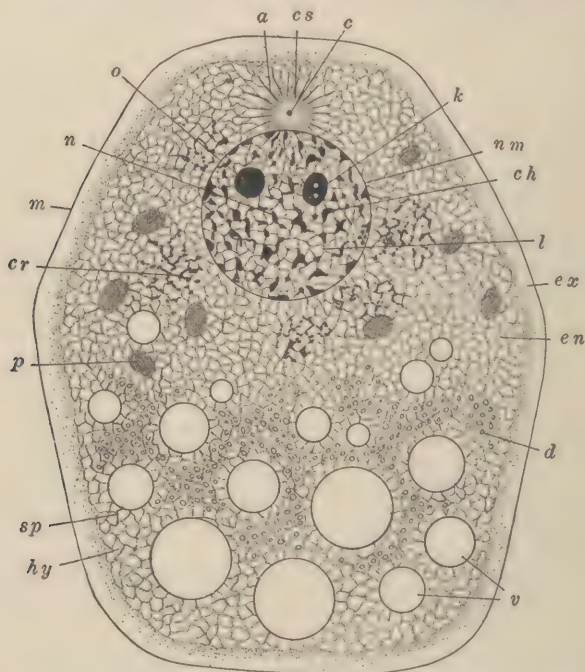

Fig. 1. — Diagram of a typical cell. From Kellicott (General Embryology). *a*. Asters. *c*. Centrosome (centriole). *ch*. Chromatin. *cr*. Chromidia. *cs*. Centrosphere. *d*. Deutoplasmic granules. *en*. Endoplasm. *ex*. Exoplasm (cortical plasm). *hy*. Hyaloplasm. *k*. Karyosome. *l*. linin network. *m*. Cell membrane. *n*. Nucleus. *nm*. Nuclear membrane. *o*. Nucleolus. *p*. Plastids. *sp*. Spongioplasm. *v*. Fluid vacuoles (metaplasm).

the light of present knowledge a more inclusive and accurate description may be formulated thus: a cell consists of nuclear material and the protoplasm within its sphere of influence.[1] Such a definition is preferable to the older one because it takes account of certain proto-

[1] Even the second definition does not include such specialized cells as the enucleated red blood corpuscles of mammals.

plasmic masses containing several nuclei, and termed *syncytia,* as well
as of cells whose nuclear substance is diffuse. The protoplasm in
its unstained condition is a grayish substance which may appear granu-
lar, recticular, or alveolar (see below), depending on the type of cell
and the circumstances under which it is being observed. Further-
more, the content of the nucleus itself is really only another sort
of protoplasm differing somewhat in its composition and organiza-
tion from that outside. For this reason it is often customary to dis-
tinguish the protoplasm within the nucleus as *karyoplasm* and that
surrounding it as *cytoplasm.* Lastly the nucleus and the cell as a
whole are usually each enveloped in a membrane. The membrane of
the cell, however, is not infrequently lacking or invisible, while in
other cases there may be more than one. Likewise the nucleus may
lack a membrane, and in some instances the nuclear material may
even be widely scattered throughout the cytoplasm. This last condi-
tion, however, is relatively exceptional, and is found more particularly
among certain of the Protozoa and the Bacteria.

The Cytoplasm. — Considering this part of the cell first, we find
that it in turn is generally divisible into *active* or " living " cytoplasm
and *metaplasm.* The former is in general the more homogeneous and
in the case of granular protoplasm the finer grained substance; as
suggested, it is supposed to represent the truly living material of the
cell. The metaplasm, on the other hand, consists of the larger granules,
oil drops, and vacuoles of various sorts. These may represent food
materials (*deutoplasm*) or excretory substances, and in either case
are regarded as relatively inert and " dead." It must, nevertheless,
be strongly emphasized that it is impossible to draw any hard and
fast line between those substances which are active cytoplasm and
those which are mere metaplasm. As a matter of fact one shades in-
sensibly into the other, and the distinction is one of descriptive con-
venience rather than of reality. As regards the distribution of the
above types of cytoplasm we find that there is considerable variation.
In the general type of cell under discussion, however, the metaplasmic
material may be imagined as scattered rather indiscriminately.

Let us now turn to a more detailed consideration of the so-called
active cytoplasm, as indicated by the terms granular, recticular, and
alveolar.

Some of the earliest students of cells, including Schultze, regarded
the essentially living protoplasm as made up of a viscid liquid called

hyaloplasm, filled with fine granules termed *microsomes.* This conception was largely due to the fact that they studied living cells, in which this appearance is not uncommon.

Later workers, however, such as Strasburger, Van Beneden, and Leydig, made their observations upon killed tissue in which the protoplasm had been coagulated and stained. To them the supposedly active substance appeared like a network or reticulum whose interstices contained the liquid of Schultze. Thus Leydig termed
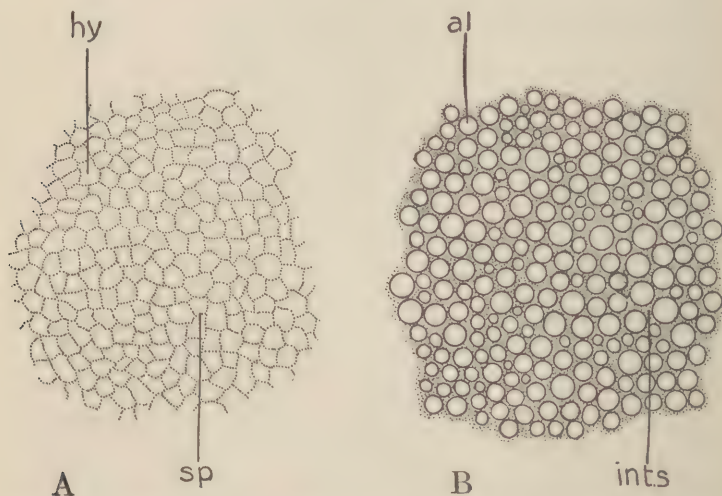

Fig. 2. — Diagram representing, A, reticular, and B, alveolar protoplasm. *al.* An alveolus. *hy.* Hyaloplasm. *int.s.* Interalveolar substance. *sp.* Spongioplasm.

the reticulum itself *spongioplasm* and for the liquid retained the word hyaloplasm (Fig. 2, A).

Subsequently to this (1890), Bütschli proposed the *alveolar* conception of protoplasm. He too studied living cells, mostly Protozoa, and to him the active substance appeared like bubbles or globules of one liquid packed closely together and suspended in another; i.e., it had the character of an emulsion. The globules he called *alveoli,* and the material between them the *interalveolar substance.* The latter, he admitted, often contained microsomes (Fig. 2, B).

Finally as regards the present view of the active cytoplasm the following may be said: it seems likely, as already intimated, that differ-

ent cells, or even the same cell at different times and under different conditions, exhibit all of the above types of structure. More recently, two other types of granules have been noted: one termed *mitochondria,* may be identical with the microsomes; the other, *chromidia,* seem to be chromatin grains extruded from the nucleus.

There is next to be mentioned a rather important cell organ which usually lies in the cytoplasm near the nucleus, and which is often visible during the resting stage. It is a minute granule, or group of granules, which stains darkly with nuclear stains, and is termed the *centrosome* or *centriole.* Immediately surrounding it there is usually a somewhat clearer cytoplasmic area which is known as the *attraction sphere* or *centrosphere.* The structure as a whole is intimately connected with the division mechanism of the typical cell, and also, as will appear later, with the special motor apparatus of the male germ cell.

Besides the centrosome, whose occurrence is very widespread, there also exist in the cytoplasm of certain cells two other types of cell organ. The first of these types consists of bodies of various shapes, sizes, and colors, often apparently located at random. They are termed *plastids,* and are most frequently found in plant cells, where they contain chlorophyl (*chloroplastids*) and are active in the formation of starch. In other kinds of cells other varieties of plastids are present, differing from the chloroplastids not only in composition and color, but also, as would be expected, in the substances which they produce. In all cases, however, these bodies are to be distinguished from typical metaplasmic material because of their activity and also because they are thought in many instances to be self-propagative. The second type of cytoplasmic organ is the *contractile vacuole.* Vacuoles of this sort differ from those of a more strictly metaplasmic nature in respect to their contractile character, their constancy of occurrence in those cases where they occur at all, and in many instances in their constancy of position. Furthermore, they are said in certain cases to reproduce, like the plastids, by fission. They are found most frequently among the Protozoa.

Lastly there are often used in connection with the cytoplasm the terms *exoplasm* and *endoplasm.* The former naturally indicates the cytoplasm close to the surface, and the latter refers to that nearer the center. Frequently, however, it is impossible to distinguish sharply between the kinds of protoplasm in the two localities, and when it is, the kinds in different types of cells are found to differ widely in their

relative appearance. Thus these terms are indicative of the location in which a substance is found rather than of its character.

This brings us to the covering of the cell, which may be essentially of two sorts. Either it is a very thin layer of the exoplasm itself, the *pellicle* or *plasma membrane*, or it is a secretion from this exoplasm, a " *true membrane*." Frequently, as in the sea urchin egg, the former is transmuted into the latter so that the distinction is not fundamental.

The nucleus. — Considering now the nuclear protoplasm or karyoplasm, it is found to resemble somewhat one type of cytoplasm: i.e., it consists essentially of a reticulum and a liquid ground substance. However, whether or not these elements are fundamentally identical with the corresponding elements of the cytoplasm is in considerable doubt. At all events they have received different names, the fibers of the nuclear reticulum being termed *linin* and the ground substance *nuclear sap*.

Upon the side of radical differences it appears that the nucleus varies from the cytoplasm markedly in the virtual lack of one substance and in the presence of another. Thus there is practically no nuclear material which is obviously metaplasmic. There is on the other hand the presence of a material which, though it sometimes occurs elsewhere, is essentially characteristic of the nucleus and is termed *chromatin*. As regards its origin, it is thought that this substance may be differentiated out of the linin network itself. However that may be, during the period which we are describing it generally exists as minute granules embedded along the linin fibres, or gathered into larger masses called *chromatin nucleoli* or *karyosomes*. In its chemical composition, chromatin is known to be especially rich in a complex phosphorus-containing acid, known as nucleinic acid. Also, probably on account of this acid, the chromatin possesses a peculiar affinity for haematoxylin and certain basic tar-colors. Thus in properly stained preparations it is always characteristically colored, and to this fact it owes its name. In unstained material on the other hand it is quite colorless, thus causing the nucleus to appear lighter than the surrounding protoplasm. As will be observed later, this chromatin is probably the most important substance which the cell contains, and is the vital center of all its activities.

One other body which the nucleus may contain is another type of nucleolus known as a *plasmosome,* or true nucleolus. It differs from the chromatin nucleolus, or karyosome, in that it has no affinity for the

special chromatin stains. Instead it takes the acid colors which stain the cytoplasm. It does not always occur and its function is uncertain.

Finally, as already noted, the nucleus like the cell is almost always surrounded by a more or less definite membrane. The true nature of this membrane is somewhat in doubt, but it is probably of the same fundamental nature as the nuclear reticulum.

THE CELL IN DIVISION

There are two types of cell division, one of which is termed *amitosis* and the other *mitosis*.

Amitosis. — This form is relatively rare and is believed by many to occur only in cells that are degenerating or in some other respect abnormal. In any event the process is very simple and will be described here only briefly. The nucleus does not undergo any apparent internal change but simply constricts itself into two approximately equal parts. This is followed in most cases by a similar division of the cytoplasm in such a manner that each portion contains a part of the original nucleus. In some instances, however, which can hardly be termed real cell division, only the nucleus thus divides, thereby starting the production of a syncytium.[2]

Mitosis or Karyokinesis. — This is the more usual method of cell division and is far more complicated than that just described. There are numerous minor variations in the process in different kinds of cells, but in general it involves certain stages or phases characterized by the following events:

I. The Prophase. — The granules of chromatin which are embedded in the linin, become more easily stained and are soon concentrated into a chromatin thread, frequently continuous, and known as the *spireme*. The constituent granules of this thread, which remain visible for a time, are often termed *chromomeres* (Fig. 3, II). Soon, however, the spireme begins to shorten and thicken, and presently is broken up into segments (Fig. 3, III). These segments are called *chromosomes*, and there is normally a specific number for every species of plant and animal. The only regular variation in this number within a given species is one which will be described below in connection with the consideration of sex. As preparation for division goes on, the relatively slender chromosomes grow still shorter and thicker

[2] It is not to be understood that syncytia arise only as a result of amitosis.
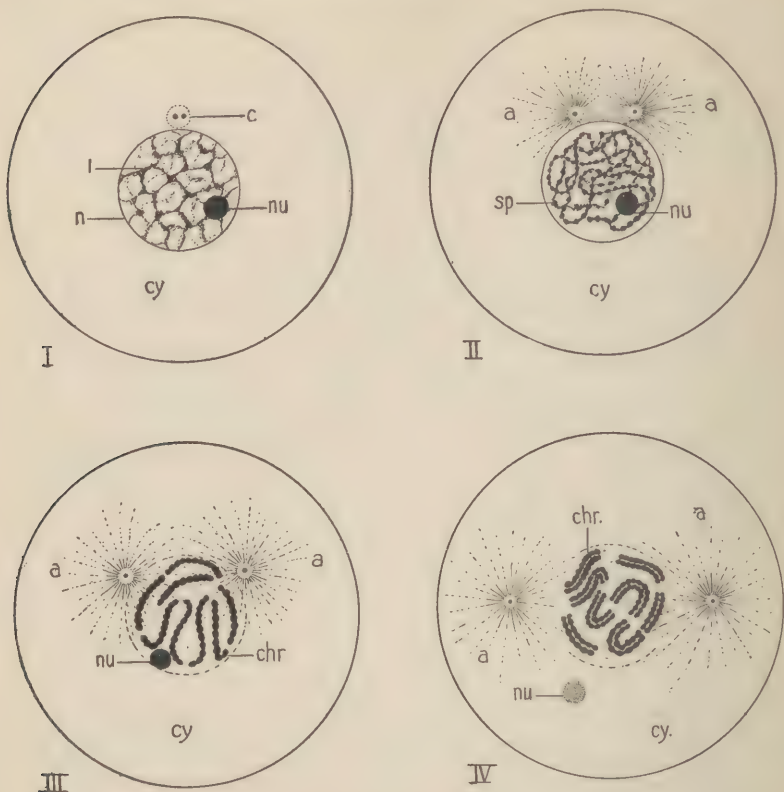
Fig. 3. — A generalized diagram of the resting cell and three stages in the *prophase* of mitosis. I. The resting cell. II. An early prophase of mitosis, showing the chromomeres arranged in a continuous spireme. The centrosomes have separated, and about the centrosphere of each an aster has formed. Between the centrospheres an incipient spindle is suggested. III. A later prophase in which the spireme has thickened somewhat and has become divided transversely into chromosomes. The asters have separated still further, and the nucleolus is about to pass out through the disappearing nuclear membrane. IV. A still later prophase. The chromosomes, continuing to give evidence of their constituent chromomeres, have shortened still more and are split lengthwise. The centrosomes surrounded by their centrospheres and asters have reached opposite sides of the nucleus. The membrane of the latter has almost disappeared and the nuclear region is now crossed by the spindle connecting the centrospheres. The nucleolus is vanishing in the cytoplasm.

a. Aster surrounding centrosphere and centrosome. *c.* Centrosphere containing the divided centrosome. *chr.* Chromosomes in the process of forming. *cy.* Cytoplasm. *l.* Linin network of resting nucleus containing granules of chromatin. *n.* Nucleus. *nu.* Nucleolus. *sp.* Spireme.
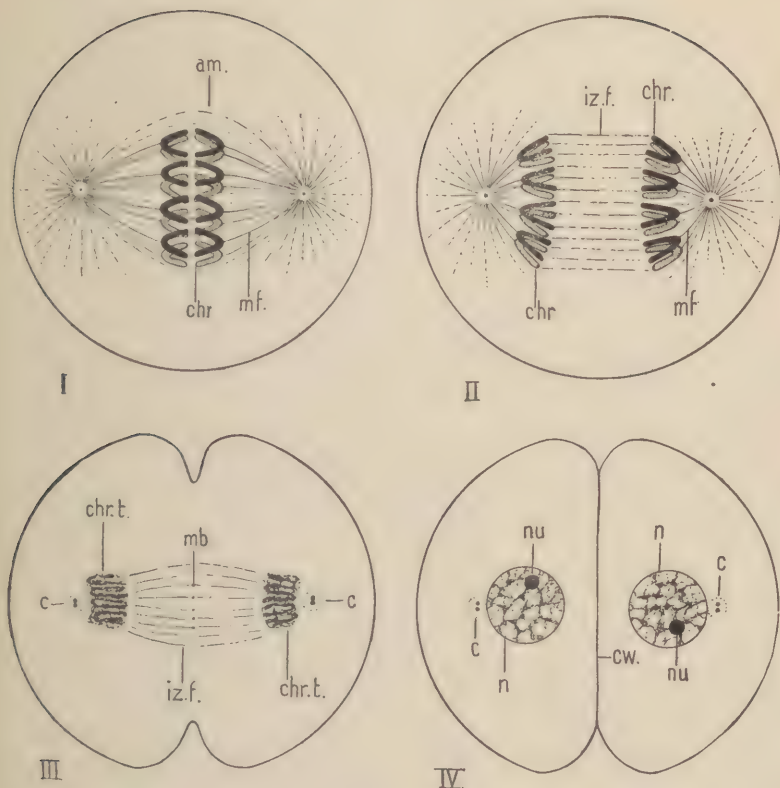
Fig. 4. — A generalized diagram showing the continuation and completion of the process of mitosis whose initial stages are indicated in Fig. 3. I. The *metaphase*. In this stage the two new sets of chromosomes resulting from the longitudinal split of each original chromosome have just separated from one another and are moving toward their respective asters. The amphiaster is completely formed, and a mantle fiber is attached to each chromosome, whose chromomeres are no longer visible. The point of attachment is represented here as being at the middle of each chromosome, so that the latter has been pulled out into a V shape. This is not always the case. II. The *anaphase*. The chromosomes of each set are approaching the region of the respective centrospheres, and between the former stretch the interzonal fibers. III. The *telophase*. The cytoplasm of the cell is beginning to divide, and the chromosomes in each portion are being resolved once more into a reticulo-granular condition. The centrosome inside each centrosphere has started to divide preparatory to the next division of the cell, and upon the interzonal fibers some mid-body granules are visible. IV. The *divided cell*. The cytoplasm of the cell has completed its division, and the two new nuclei have resumed the resting condition.

am. Completed amphiaster, consisting of the two asters and the spindle between them. *c.* Centrosphere containing the centrosome in process of division or divided. *chr.* Chromosomes completely formed and in process of separation. *chr.t.* Chromosomes of the telophase becoming resolved into the reticulo-granular condition. *cw.* New cell wall between the divided parts. *iz.f.* Interzonal fibers. *mb.* Mid-body granules. *mf.* Mantle fibers. *n.* Nucleus. *nu.* Nucleolus.

until they appear like rods, or even blocks or spheres. Frequently also, during this stage, each rod appears longitudinally split (Fig. 3, IV). Indeed in many cases this split is visible in the unsegmented spireme and seems quite clearly due to a splitting of the chromomeres themselves. In other instances the actual division is not evident until the following phase. In any event, the result is that eventually each chromosome becomes composed of two equal halves. During the above processes the plasmasome, if present, disappears. The karyosome, on the other hand, may partake in the formation of the chromosomes, or it may later be cast out into the cytoplasm and lost.

Meantime certain changes have occurred outside the nucleus. The centrosome, if it did not divide into two parts immediately after the preceding cell division, now does so. The attraction sphere then likewise divides, and about each centrosome and its attraction sphere, fibre-like rays begin to appear extending out into the cytoplasm. Each such center together with its rays is termed an *aster*. The two asters now move apart, and shortly take up positions on opposite sides of the nucleus.

II. The Metaphase or Mesophase. — At about this point, sometimes earlier, sometimes later, the nuclear membrane disappears and the chromosomes become arranged either in a plate (*equatorial plate*) or a ring at right angles to the axis which connects the two centrosomes. The astral rays between these bodies form a kind of spindle (*mitotic spindle*) around or across whose equator the split chromosomes are grouped. This whole affair apart from the chromosomes is then termed an *amphiaster*. At this time some of the fibres of the spindle seem to become attached to the chromosomes, the fibres thus functioning often appearing heavier than the others and being known as *mantle fibres* (Fig. 4, I).

III. The Anaphase. — The halves of each chromosome now begin to separate from one another, thus producing two chromosomal groups each having the same number of chromosomes as the original nucleus. Each of these groups then moves toward the center of one of the asters. Whether this movement is due to the contraction of the mantle fibres or to some other cause has long been a matter of controversy. It now appears fairly certain that whereas such a contraction may have something to do with the movement, other unknown factors are also largely involved. Indeed in many cases it is doubtful if the contractility of the mantle fibres has anything to do with the process. At any rate, as the separation occurs those spindle fibres which do

not appear directly attached to the chromosomes remain as a fibrillar bundle connecting the two groups. They are called the *interzonal fibres* [3] (Fig. 4, II).

IV. The Telophase. — The two groups of chromosomes presently reach the vicinity of the center of their respective asters. Each group then begins to resolve once more into the reticulo-granular condition typical of the resting stage. The exact method by which this is accomplished varies in different kinds of cells, but the result in every case is a new nucleus, surrounded by a nuclear membrane. As a general rule the centrosome which was at the center of each aster moves aside somewhat as the new nucleus is formed, and thus comes to lie just outside the nuclear membrane, surrounded by the usual attraction sphere. The latter is now all that is left of the elaborate aster, which it sometimes resembles in the possession of very delicate rays. Within this sphere the centrosome may divide immediately into two parts preparatory to the next cell division, or it may remain single throughout the resting stage (Fig. 4, III, IV).

Meanwhile during the nuclear telephase the cytoplasm also divides. This occurs by means of a constriction passing around the original cell at right angles to the spindle and in the region of its middle. Presently, like the rays of the asters, the interzonal fibres disappear and the process of constriction cuts the cell in two. Usually before this occurs, however, the above fibres show at their equators more or less deeply staining thickenings which often give rise to what is termed the *cell-plate* or *mid-body*. In animal cells this frequently amounts to only a few granules and disappears with the fibres. In plants, however, it nearly always forms a well-developed plate through whose plane the cell is divided, each half of the plate going to form the new cell wall. Figure 5 illustrates the various division stages just described as they occur in a species of salamander.

The Significance of Mitosis. — In considering the meaning of mitosis it should be noted that the division of the cytoplasm is often quite unequal, depending on conditions and the type of cell. It has been seen on the other hand that the division of the chromatin in the form of chromosomes appears to be quantitatively exactly equal. The question immediately arises, however, as to whether such an elaborate

[3] It should be mentioned in this connection that in the cells of most higher plants and in those of some animals no centrosome or aster is present, though the interzonal fibres appear as usual. In such cases it is evident that there can be no question of the separation of the chromosomes by fibrillar contractility.
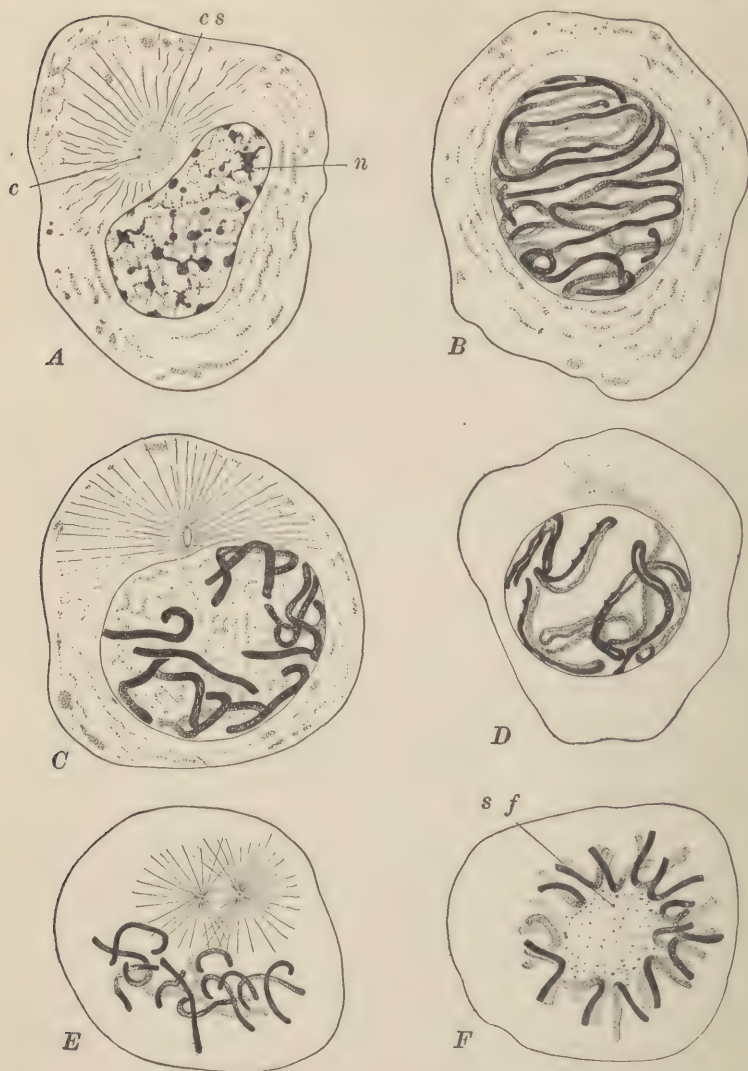
Fig. 5. — Mitosis in cells of *Salamandra maculosa*. From Kellicott (General Embryology). After Prenant and Bouin. D. H. Primary Spermatocytes, others spermatogonia. A, B, C, x 1000, others, x 800. A. Interkinesis or resting stage. B. Early prophase; spireme continuous. Centrosomes omitted. C. Prophase; spireme seg-

mented into chromosomes. Centrosomes commencing to diverge; spindle forming.
D. Longitudinal splitting of chromosomes. E. Disappearance of nuclear membrane;
continued divergence of centrosomes and asters. F. Mesophase; formation of equatorial
plate. Polar view. Chromosomes V-shaped. G. Same in side view. Only a few of the
chromosomes are shown. H. Late mesophase; daughter chromosomes diverging, still
united at ends. I. Anaphase; continued divergence of chromosomes, now entirely sep-
arated. J. Late anaphase; complete divergence of chromosomes. Spindle breaking
down, asters disappearing. K. Telophase; beginning of reconstruction of daughter
nuclei. Chromosomes disintegrating. L. Late telophase; division completed. Nuclei
reconstructed; centriole divided; cell walls completed. Nuclear membrane forming.
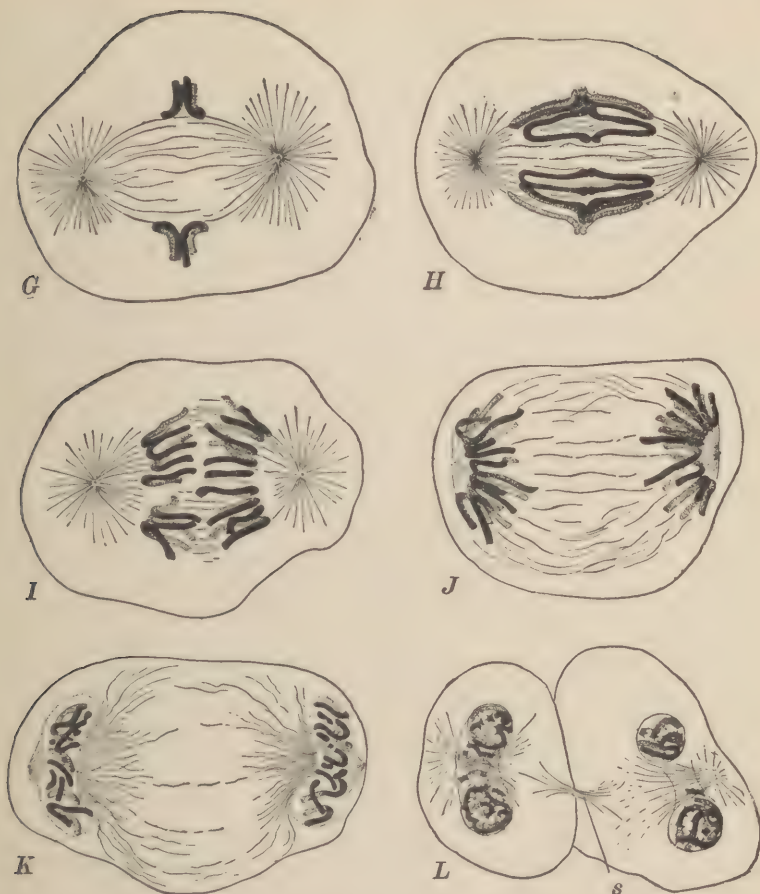
c. Centrioles. cs. Centrosphere. n. Nucleus. s. Spindle remains. sf. Spindle
fibers cut across.

mechanism as that of mitosis would be necessary to effect a mere quantitative equality of division. Is it not likely that a qualitative difference exists in the chromatin, and that the significance of the above mechanism lies partly therefore in the securing of an accurate and orderly distribution of the qualities involved? It is at present impossible to answer this question with certainty so far as the evidence obtained from direct observation of the chromosomes is concerned. The following considerations, however, serve to throw some light on the problem.

Whatever may be said regarding their finer constitution, the chromosomes in a given species of organism usually differ from one another as to form and size. Secondly, this difference is constant at each appearance of the chromatin in this condition. Finally the division of the chromosomes, involving, as it often does, a longitudinal split, is such as always to result in apparently equal and similar halves. Nevertheless, these facts in themselves do not prove the chromatin to differ qualitatively. Nor if this were true, do they prove that similarly appearing chromosomes always necessarily contain the same kind of chromatin. That such is the case will become evident from the following considerations.

It must be remembered that although the chromosomes are clearly visible during cell division, there always follows a resting stage during which in most instances they entirely disappear as such. On the basis of direct observation, therefore, it is quite uncertain as to whether the chromatin occurring in a chromosome of given size and shape at one division is either the same chromatin or even the same kind of chromatin that occurred in that chromosome at the close of the previous division. Indeed in some instances whatever may be the aspect of the chromatin which reappears after the intermitotic interval, it can not all be identically the same chromatin which disappeared at the beginning of this period. This follows from the fact that in some cases during the intermitotic stage a part of the chromatic substance is known to be cast out of the nucleus and apparently transformed into cytoplasm (Fig. 26). On the other hand although the identity of the actual chromatin in a given chromosome may not remain constant from one division to another, it seems certain that some element connected with it must do so. Otherwise it is impossible to account for the above mentioned constancy of the chromosomes as to size and form. It has therefore been held that what really persists is some

invisible organization of material not necessarily to be identified with the chromatin itself. This organization is then capable at each cell division of building up chromosomes similar, at least in appearance, to those which disappeared at the end of the preceding division. That is, there is a sort of genetic continuity of the morphology of a specific chromosome from one mitosis to the next, or in other words, the arrangement of its material seems identical each time the chromosome appears as such. This, however, is all that the mere microscopic examination of normal cells will demonstrate. Hence, as suggested above, the problem as to the possible existence and genetic continuity of finer qualitative differences among the chromosomes remains from this standpoint unsolved.

Although direct observation of the chromosomes is thus insufficient to prove them possessed of persistent qualitative differences, there is a great mass of very cogent evidence of an entirely different sort which lends strong support to this view. This evidence is obtained (1) from certain experiments in abnormal fertilization, and (2) from the field of *genetics;* i.e., the study of heredity.

Evidence from Experiments in Fertilization. — Before coming to the actual experiments in this connection it will be necessary to say a word more regarding the appearance of the chromosomes. It has already been noted that the chromosomes in any cell of a given species of animal differ from one another in appearance. It must now be added, however, that they do not all differ; that they occur, in fact, in pairs whose members appear identical. The chromosomes of such a pair do not, it is true, always lie next to one another, but because of their form and size they are, nevertheless, recognizable as mates. Thus it appears that with certain exceptions to be noted later, there are present in each cell two identical chromosomal sets. All the cells of the body, however, have arisen during the course of the animal's development by mitotic division extending back to the fertilized egg or zygote. This then means that the zygote also contained two sets of chromosomes. One of these sets the egg possessed before fertilization; i.e., its union with a male germ cell or sperm; and the other it obtained as a result of that process. The details of these phenomena will be described later in connection with the study of the germ cells and their behavior. The only point which need now be noted is the fact that each chromosomal set in every cell of the body was thus derived from one of the parents of the animal in question (Fig. 6).

Fig. 6. — Diagrams illustrating the distribution of the paternal and maternal chromosomes during cleavage. From Kellicott, (General Embryology.) A. The fertilized egg or zygote, containing the egg nucleus, ♀, and sperm nucleus, ♂, side by side previous to the disappearance of their respective nuclear membranes. Each nucleus, it will be noted, contains an identical chromosomal set, distinguished for convenience by printing that of the sperm nucleus in black. B. First cleavage figure, showing all the chromosomes on the spindle and each one divided. C. Two-cell stage, each nucleus containing two sets of chromosomes, derived by division from each of the parents. D. Second cleavage, in which each cell is divided as in B. E. Four cell stage, showing four cells, each with a nuclear content similar to that of the nuclei in C.

With these facts in mind it is now possible for the student to appreciate
the significance of the experiments indicated. Only two will be cited
as of especial importance.

(1) Baltzar crossed two genera of sea-urchin, *Strongylocentrotus*
and *Sphærechinus*. When the former genus was the male and the
latter the female, the offspring were intermediate in their characters.
When, however, the cross was reversed; i.e., Sphærechinus male to
Strongylocentrotus female, the characters of the larvæ were entirely
maternal. Examination then revealed the fact that in the latter
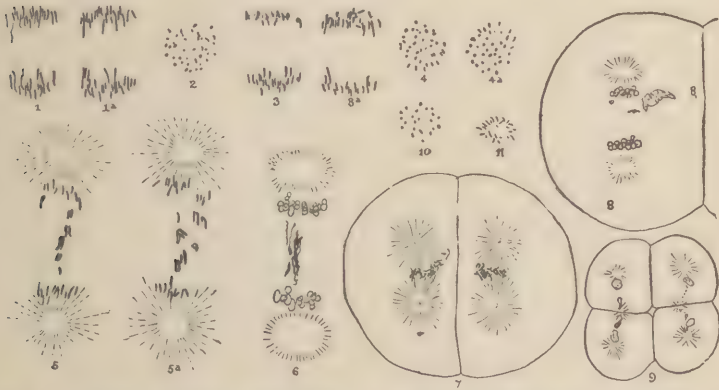
Fig. 7. — From Morgan (Mechanism of Mendelian Heredity). After Baltzer
1 and 1a, chromosomes in the first normal cleavage spindle of Sphærechinus; 2, equa-
torial plate of two-cell stage of same; 3 and 3a, spindles of two-cell stage of egg of
hybrid of Sphærechinus by Strongylocentrotus; 4 and 4a, same, equatorial plates;
5 and 5a, hybrid of Strongylocentrotus by Sphærechinus, cleavage spindle in telo-
phase; 6, next stage of last; 7, same, two-cell stage; 8, same, later; 9, same, four-
cell stage; 10, same, equatorial plate in two-cell stage (22 chromosomes); 11, same,
from later stage, 24 chromosomes.

case for the most part only the Strongylocentrotus chromosomes sur-
vived, the majority of the chromosomes from the Sphærechinus sperm
perishing at the first cell division. This experiment, therefore, would
seem to indicate that it is the chromosomes which in some way deter-
mine the generic characters of an organism, and hence that the
chromatin of which they are composed is presumably qualitatively
different (Fig. 7).

(2) Boveri experimented with sea-urchin eggs that had been fer-
tilized by two sperm of the same genus. In such cases he found that
at the first cell division three or more asters formed, making it im-

possible for all of the cells to obtain a complete set of chromosomes
(Figs. 8 and 9). Now in cases of normal fertilization in these animals,
it was known that if the cells were separated in the four-cell stage,
each would develop into a normal larva. In his experiment, there-
fore, Boveri shook the cells apart and found that in this case only
a few developed normally. It appeared, nevertheless, that the num-
ber of normal individuals corresponded approximately to the number
of cases in which, according to chance distribution, at least one
complete set of chromosomes should be present. Furthermore, the
cells having the larger nuclei furnished a much greater proportion of
normal larvae. But the size of the nucleus is known to be roughly
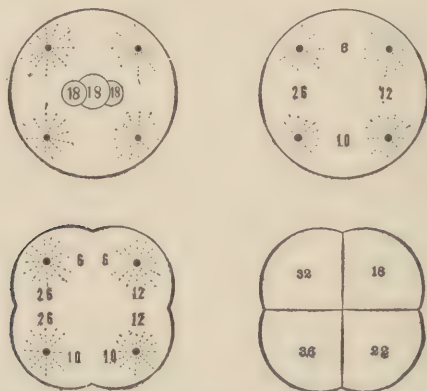
Fig. 8. — Dispermic fertilization of egg of sea urchin. From Morgan (Mechanism of
Mendelian Heredity). The four centrosomes cause an unequal distribution of the
fifty-four chromosomes, leading at the first division to four cells which contain
different numbers of chromosomes.

proportional to the number of its chromosomes. It was thought fair
to infer therefore that the greater the number of chromosomes a cell
contained in this case, the greater was its chance of normal develop-
ment. It was also clear, however, that the greater the number of
chromosomes, the greater the chance that at least one complete set
was present.

From this experiment, therefore, the following inferences may be
drawn. Not only is the chromatin qualitatively differentiated, but
its distribution is such that at least one complete set of chromosomes
must be present in order to supply all the qualities needed to secure
normal development. That is to say, the chromosomes not only

differ in form and size but also apparently in the kind of chromatin they contain. It is to be noted, however, that even this is not proof that the kind of chromatin in a *particular* chromosome, or in a particular *pair* of chromosomes, is always of the same sort.

Evidence from Genetics. — The evidence in this field is so extremely voluminous and the subject so complex, that it will be impossible in a book of this type to treat the matter at all fully. Also

Fig. 9. — From Morgan (Mechanism of Mendelian Heredity). After Boveri. Diagram to show five combinations of chromosomes resulting from the first division of dispermic eggs, in which either each cell gets one complete set of chromosomes, *a;* or three cells get a full set, *b;* or two cells, *c;* or one cell, *d;* or none of the four cells, *e;* get a full set.

until the student has become familiar with the phenomenon of maturation and the behavior of certain chromosomes he can not well appreciate the true significance of the genetic arguments. The details of these arguments will therefore be postponed until the subject of maturation has been discussed, and it must suffice for the present to state the matter in this way. Reasons have already been given for assuming that the chromosomes are chiefly responsible for the generic characters. Let it now be further assumed that the chromosomes

also bear " factors " called *genes* or *determiners* (i.e., different kinds of chromatin) which control all the characters of an organism, and secondly that the location of these genes is constant, at least as to the pair of chromosomes in which they occur. This latter point, it will be recalled, is one which the facts cited in the preceding paragraph did not prove, but in support of which strong evidence will later be presented. Anticipating this evidence for the time being, however, it may now be stated that, on the basis of the above assumptions it has been found possible to explain in a remarkably logical and convincing manner a great mass of hereditary phenomena. Furthermore, these phenomena cannot be so satisfactorily or fully explained on any other hypothesis so far advanced. There is, therefore, extremely good scientific ground for the assumptions.

General Conclusions. — Concerning the general significance of mitosis, therefore, the matter may now be summed up as follows: There seems to be very good evidence that the chromatin differs qualitatively, and that the chromosomes of a given pair, as indicated by form and size, always contain chromatin of the same sort. Also it appears that whatever these differences are, they are of some vital significance in controlling the structure of the cells and the organism of which the latter are a part. Furthermore, there is the fact that mitosis results in the apparently equal division of the chromosomes. It seems highly probable, therefore, that the question propounded regarding the significance of mitosis may be answered in the affirmative; that is, the main function of this elaborate process is to secure a definite and accurate distribution of the chromatin with its differing qualities, in a fixed and orderly manner.

THE GONADS AND THE GERM CELLS

The *germ cells* or *gametes* are certain cells in which both cytoplasm and nucleus are specialized for the purpose of reproduction. They are thus distinguished from body or *somatic* cells which are specialized for other functions in the life of the organism. Before considering the detailed development of the germ cells it will first be necessary to give a brief general history and description of the organs in which they are finally located.

THE GONADS

The germ cells of the adult occur in organs known as *gonads,* the female gonad being termed the *ovary,* and the male gonad, the *testis.* In most true Vertebrates these are paired structures, and in the same individual both members of the pair are normally of the same sex. In their earliest condition both ovaries and testes appear alike, as
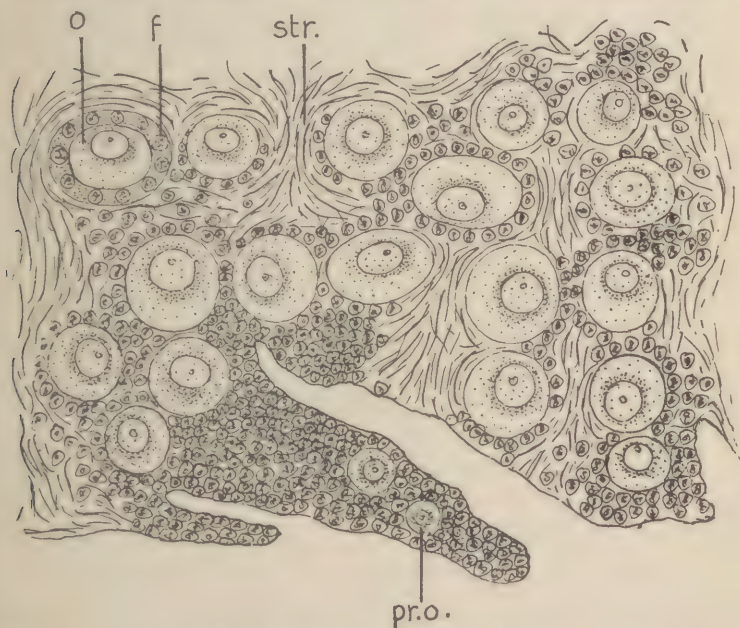
Fig. 10. — Cross section of the ovary of a fledgling of *Numenius arcuatus* 3–4 days old. From Lillie after Hoffmann. The region of the germinal epithelium is toward the bottom of the figure. *f.* Follicle. *o.* A very young ovum around which the epithelial cells have formed a definite follicle. *str.* Stroma. *pr.o.* Primitive ovum within a portion of the germinal epithelium.

a pair of ridges (the *genital ridges*) consisting largely of thickened cœlomic epithelium (the *germinal epithelium*). Beneath this epithelium there occurs a small amount of a variety of loose connective tissue known as mesenchyme, which is soon to increase and constitute the supporting element of the organ, termed the *medullary tissue* or *stroma.* Each genital ridge lies along the back on either side of the dorsal mesentery of the gut between it and the embryonic excretory

organ.   Within the germinal epithelium there presently appear certain cells which are often distinguishable from their fellows by their larger size and also by their relatively larger nuclei.   These are the primitive or *primordial germ cells* in which sex differentiation, at least as regards
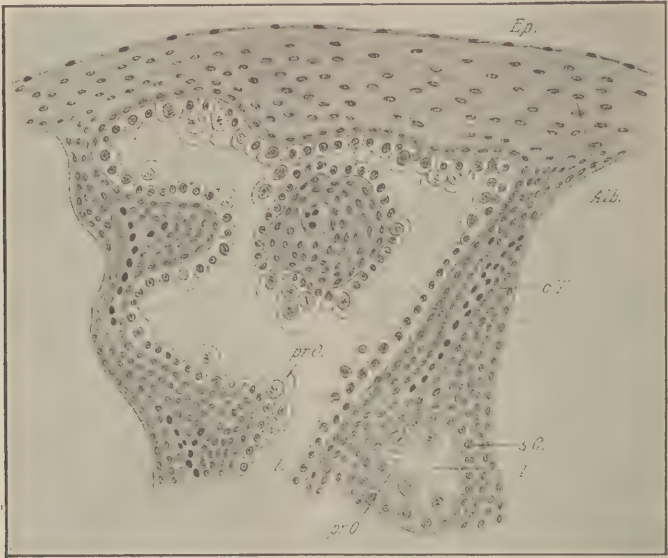
Fig. 11. — Cross section through the periphery of the testis of a just hatched Chick.   From Lillie (Development of the Chick).   After Semon.   The sexual cords have acquired a lumen, and the walls of the canals thus formed are lined within by the spermatogonia.   Next to the latter come a layer of supporting or Sertoli cells, and outside of these a thin layer of connective tissue, the theca (not labeled).   The remaining connective tissue (stroma) lying between the sexual cords (now seminiferous tubules) connects at the periphery of the testis with the special layer of connective tissue (albuginea) which covers the entire organ beneath the thin outermost layer of germinal epithelium.

Alb. Albuginea.   *c.T.* Connective tissue of the stroma, or septulæ testis.   *Ep.* Remains of the germinal epithelium now forming the outermost or serous covering of the testis.   *l.* Lumen of the sexual cords.   *pr.O.* Spermatogonia.   *s.C.* Sexual cord, lined by supporting cells and spermatogonia.

the cytoplasm, is not yet apparent.   The origin and later development of these cells will be discussed after completing our description of the gonads.

The Ovary. — In the case of the ovary as the germinal epithelium gradually increases in thickness it is in some instances divided by the stroma into columns or strands termed the *ovigerous cords*.   In any

event, during the course of growth, groups or nests of the epithelial cells, each containing a primitive ovum, become scattered about throughout the connective tissue. Each germ cell then proceeds to develop as such, while the epithelial cells which surround it, known as its *follicle* (Fig. 10), serve to convey it nutriment.

The Testis. — Within the young gonad which is to become a testis there develop throughout the stroma, strands of tissue, in this case termed *sexual cords*. Though their origin in some instances is doubtful, they apparently arise, like the ovigerous cords, from the germinal epithelium. Whatever their origin, however, they presently become filled with the germ cells which seem to migrate into them. These cords then become tubular, and the tubes are lined by the germ cells, either arranged in layers or enclosed in cysts (Amphibia). The walls of the cysts, if there are any, or the epithelial walls of the tubes are homologous in function to the follicle cells of the ovary; i.e., they bring nutriment to the growing germ. Such cells in this case are often termed *supporting cells*, or *Sertoli cells*. Externally each tube is covered with a thin layer of connective tissue termed the *theca*, and the whole is known as a *seminiferous tubule* (Fig. 11).

A more detailed description of the development and structure of a typical vertebrate ovary and testis will be found in our treatment of this subject in connection with the Chick. Likewise short discussions of these organs are included in the accounts of the other animals to be studied. With this as an introduction the student is now prepared for a description of the history of the actual germ cells.

THE GERM CELLS

The Origin of the Primordial Germ Cells. — It was originally believed that the primordial cells of the gonads arose through the modification of some of the cells of the germinal epithelium. It is still thought that in many cases at least the later germ cells do so arise, and hence the term, germinal epithelium, continues to be applicable to this tissue.

Recent research on various Vertebrates, however, has revealed the following curious fact: The earliest germ cells to be found in the germinal ridges do not originate there at all; instead they have migrated thither usually through the mesentery of the gut (Fig. 12).

Their actual place of origin is somewhat uncertain, but in some instances (Reptile, Bird) these cells are known to become distinguishable as such even out in the endoderm of the yolk sac. This last mentioned organ is an appendage of the embryonic gut which in many cases contains the nourishment of the young animal in this stage of its development. The endoderm is a layer of cells which lines the
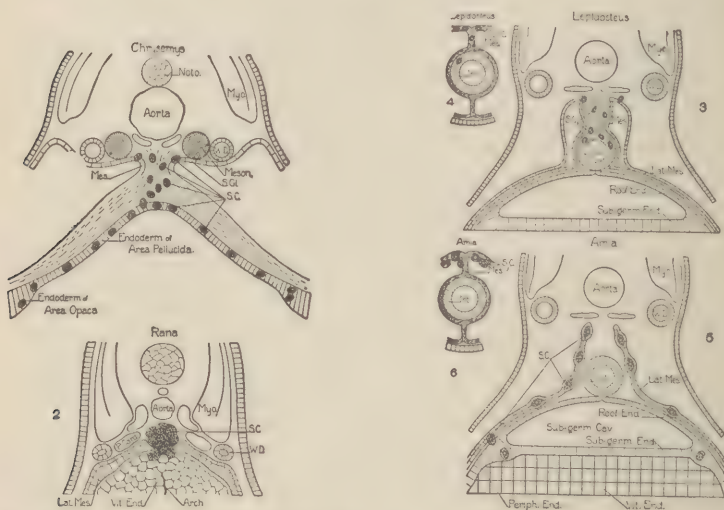
Fig. 12. — From Morgan ("Heredity and Sex." Published and copyrighted by the Columbia University Press). After Allen. Origin of germ-cells in certain Vertebrates, viz. Turtle (*Chrysemys*), Frog (*Rana*), Gar-pike (*Lepidosteus*), and Bow-fin (*Amia*). The germ-cells are seen migrating from the digestive tract (endoderm). *End.* Endoderm in various localities. *Int.* Intestine. *sc.* Sex (germ) cells. *s.gl.* Region of the gonads.

gut and sac; its history will be given in subsequent chapters. Let us now turn to a consideration of the structure and development of a typical female and a typical male germ cell.

The Ovum. — The fully developed female germ cell is termed the *ovum*. The ova of different Vertebrates vary widely in size, in the amount and arrangement of their deutoplasm, and in their coverings. They are uniform, however, in their relatively large size and inertness as compared with the male reproductive cell (Fig. 13). They also resemble both the latter and each other in one particular; i.e., the behavior of their chromatin. This latter point involves a rather complicated process comprising among other things two cell divisions

of a special type, the entire phenomenon being termed *maturation*. Inasmuch as this process is not only complicated, but also of great significance, it will be considered later under a separate heading. The remaining features may now be brought out more fully by a generalized history of an ovum.

It has already been noted that the primordial germ cells which migrate into the germinal epithelium are not readily distinguishable as to sex, at least as regards their cytoplasmic morphology. Their
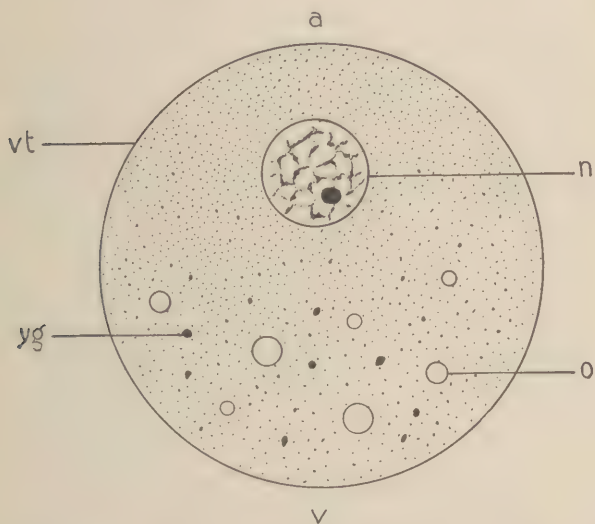
Fig. 13. — Generalized diagram of a slightly telolecithal egg ready for fertilization. The only membrane represented here is the vitelline. *a*. Animal pole. *n*. The nucleus containing a nucleolus and a linin network along the fibers of which chromatin appears. *o*. An oil vacuole. *v*. Vegetal pole. *vt*. Vitelline membrane. *yg*. A yolk granule.

male or female character becomes apparent, however, as the gonad develops and they become distributed through the stroma of the ovary, or take their places in seminiferous tubules as the case may be.

In the former instance which is now under consideration the young female germ cells in and near the epithelium proceed for a time to multiply quite rapidly. They do this by means of typical mitotic divisions, and during the process are known as *oögonia*. This stage of simple multiplication usually continues until about the time of the birth or hatching of the animal in which they are contained. In many

forms this process then ceases, so that at this time the animal in question contains as many — though only partially grown — ova as it will ever have.

The next period is one of growth during which the cell becomes surrounded by its follicle, and is termed an *oöcyte*.

The Nucleus. — The nucleus during this second period enlarges greatly, and is known as the *germinal vesicle*. It is relatively clear, though it usually contains a fine reticulum, and may possess one or more conspicuous nucleoli. The latter may be of either the plasmosome or the karyosome type or both, and their significance is not well understood. It probably varies in different cases. At the end, and also sometimes at the beginning of the growth period, certain changes occur in the nucleus which are connected with maturation. These will be described below.

The Cytoplasm. — Meantime the cytoplasm is increasing considerably in bulk, chiefly as a result in many cases of the accumulation of deutoplasm or yolk. This substance usually first appears in the shape of granules and droplets. Later it assumes various forms and contains a variety of chemical substances, consisting in general of proteids, nucleo-albumins, fats, carbohydrates, and certain salts. Not only does the composition of the yolk vary, but also its amount and distribution. Thus where the amount of deutoplasm is large the oöcyte becomes relatively enormous as in the eggs of Birds and some Fish. In such forms the yolk comes to be situated on one side of the ovum — the *vegetal pole*, whereas the remaining cytoplasm containing the nucleus occupies a greater or less part of the opposite side, or *animal pole*. Ova of this type are said to be *telolecithal*, and in those instances where this arrangement is most marked the relatively yolkless cytoplasmic cap at the animal pole is called the *blastodisc* (Fig. 14). In other ova, such as those of the Mammal, there is relatively little yolk and this is scattered throughout the cytoplasm. An egg of this type is termed *homolecithal*.

The manner in which the yolk originates and grows is of some interest. The actual new material for its formation is of course supplied from without, probably through the medium of the follicle cells. The organization of this material into yolk, however, often seems to take place about certain bodies known as *yolk nuclei*. The nature and even the exact origin of these bodies is rather uncertain, and indeed seems to vary in different cases. Frequently, however, they

appear as a darkly staining substance, somewhat similar to chromatin which usually originates near the nucleus, and according to some observers is actually cast out of it. Thus it is possible to regard them as a kind of metamorphosed chromatin. In any event when present they seem to exercise some influence over the building up of the nutritive material.
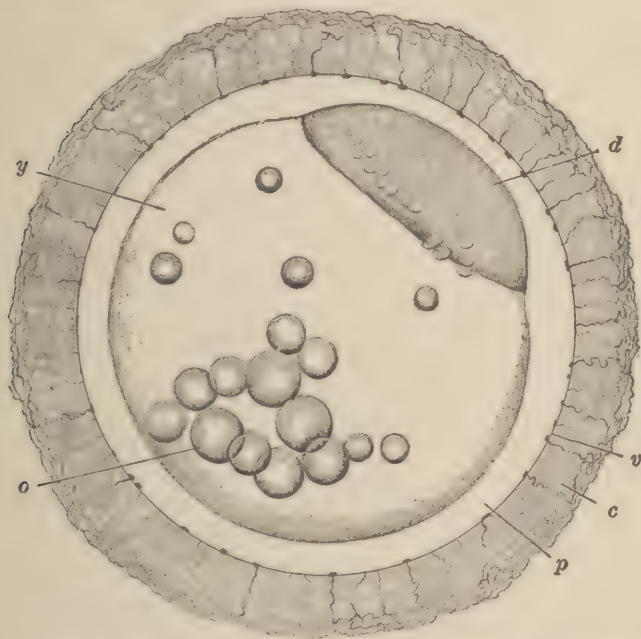
Fig. 14. — Egg of the Teleost, *Fundulus heteroclitus*. From Kellicott (General Embryology). Total view, about an hour after fertilization.
  c. Chorion. *d.* Protoplasmic germ disc or blastodisc. *o.* Oil vacuoles. *p.* Perivitelline space. *v.* Vitelline membrane. *y.* Yolk.

The Centrosome. — Concerning this body in the oöcyte there is considerable question. In some eggs the oögonial centrosome appears to persist throughout the growth period, but in many other instances this centrosome seems to vanish, a new one forming at the time of maturation. Its origin in such cases is uncertain.

The Egg Membranes. — Following growth the oöcyte, or ovum, as it may now be called, is often surrounded by as many as three different types of coverings, whose character and development are as follows. The first of these is a thin envelope immediately surrounding the egg,

termed the *vitelline membrane*. It is doubtful in the eggs of many
Vertebrates whether or not this covering is really present. When it

is present, however, it is characterized by the
fact that it is a secretion from the ovum itself.
The second covering is the *chorion,* which is
secreted by the follicle cells. It varies much
in structure and again may be entirely lack-
ing, as is probably the case in the Chick.
Finally there are frequently one or more
tertiary coverings. These may be jelly-like
as in the Frog, or one soft and the other cal-
careous as in the Bird. When present they
are always secreted by some portion of the
oviduct through which the egg must pass on
its way to the exterior.

The Spermatozoön. — The mature male
germ cell is called the *spermatozoön.* In gen-
eral it is characterized by its extremely mi-
nute size, its lack of any nutrient material
within itself, and its equipment for active
locomotion through a semi-fluid medium.
More particularly such a typical sperm con-
sists of the following main parts (Fig. 15):

I. The Head. — This is chiefly composed
of chromatin enclosed in a thin envelope of
cytoplasm. It varies greatly in shape in
different animals, but is often a more or
less ovoid disc. To its anterior end is at-
tached a tip, usually rather pointed, but also
subject to much variation in form. It is
the *acrosome* or *perforatorium.*

II. The Middle Piece. — This is a con-
venient descriptive term rather than an
accurate designation of a part which is truly
homologous in different forms. In general
it is the region immediately posterior to the
head, which, as will be noted later, usually
contains a part or all of the centrosome.
Sometimes at its anterior end is a short clear
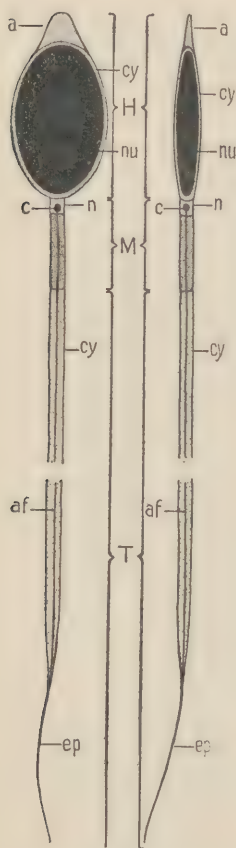
Fig. 15. — Diagram of a
generalized flagellate sper-
matozoön, showing the flat
side of the head and also
its edge.

H. Head. M. Middle piece.
T. Tail. *a.* Acrosome. *af.*
Axial filament. *c.* Centro-
some. *cy.* Cytoplasm, form-
ing an envelope for the
head and a fin for the tail.
ep. End piece. *n.* Neck. *nu.*
Nucleus.

region attaching the body of the middle piece to the head. It is termed the *neck*.

III. The Tail. — This is virtually a posterior continuation of the middle piece. It consists of a vibratile *axial filament* enveloped by cytoplasm. This cytoplasm is usually more or less in the form of a narrow fin, and may be simple or produced into curious frills or ruffles. Near the end the cytoplasm seems to be absent, and the axial filament continues a short distance as the *end piece*.

It must now be added that though the main features thus described may be regarded as typical of spermatozoa in general, there are numerous, and sometimes quite bizarre, variations. Indeed in certain cases even the characteristic flagellum is lacking, and the cell depends upon amœboid movements for its locomotion. A suggestion of the varieties of forms which occur is indicated in Figure 16.

With this idea of the general structure of a sperm in mind it is now possible to consider the stages through which such a cell passes in its development. The primordial germ cells have already been described in the study of the ovum, and it was noted that during this early period their appearance is practically alike in both sexes. This being the case, no further account of this stage is necessary in describing the history of the male cell, and we may pass immediately to the next.

By the time the male germ cells have become located in the seminiferous tubules, they have become clearly distinguishable as such. They then enter upon a period of multiplication in which they are known as *spermatogonia*. This stage corresponds in all essentials to the similar period of multiplication of the young ova (oögonia).

Following this is a time of growth which also corresponds to a period of like change among the ova (oöcytes). The cells at this time are therefore called *spermatocytes*. In this case, however, the growth though noticeable is naturally much less marked than was observed in the oöcytes, and there is, of course, no accumulation of yolk. The nucleus, nevertheless, goes through processes very similar to those which characterize the ovum at this period, at the close of which it undergoes maturation divisions. Although these divisions are fundamentally the same as those of the oöcyte, they differ in certain important details which will be considered more fully when that topic is discussed.

It will be recalled that in the case of the ovum the end of the

Fig. 16. Various types of spermatozoa. From Kellicott (General Embryology). A, B. The Teleost, *Leuciscus* (Ballowitz). C, D. The Birds, *Phyllopneuste* and *Tadorna* (Ballowitz). E, F. Two forms of the sperm of the Snail, *Paludina* (von Brunn). G. The Nematode, *Ascaris* (Van Beneden). H. The Annulate, *Myzostoma* (Wheeler). I. The Bat, *Vesperugo* (Ballowitz). J. The Opossum, *Didelphys* (Wilson). K. The Rat (Wilson). L. The Urodele, *Amphiuma* (McGregor). M. The Crustacean, *Erhusa* (Grobben). N. The Crustacean, *Inachus* (Grobben). O. The Crustacean, *Sida* (Weismann). P. The Crustacean, *Bythotrephes* (Weismann).
k. End knob. *m*. Middle piece. *n*. Nucleus. *p*. Perforatorium. *u*. Undulatory membrane. Not drawn to same scale. A–F, I–K, from Wilson.

growth period found it practically completed. This, however, is one of the points in which the spermatocyte differs strikingly from the female cell. After maturation the products of the second division are called *spermatids*, and instead of being complete they are just ready to enter upon their remarkable metamorphosis into the highly specialized spermatozoön. This process varies considerably in different animals as regards its details, particularly with respect to the exact method of formation of the middle piece and tail. For the sake of definiteness and clearness, therefore, it is necessary to confine our attention to some specific case which is fairly typical in its general aspects. The account selected is that of the metamorphosis of the spermatid in the Amphibian, *Amphiuma*, according to J. H. McGregor. The letter references throughout the description are to Fig. 17.

The Transformation of Spermatids into Spermatozoa in Amphiuma. — Here as in most cases the spermatid, at the beginning of its metamorphosis, corresponds approximately to the description of any generalized cell. It differs, however, in that its nucleus, as is usual in spermatids, is disproportionately large. Also in this particular instance the character of the centrosome is somewhat peculiar in that it lies close against the cell membrane and slightly outside of the centrosphere or *idiozome*, as it may here be termed (*A*). As has already been suggested, the head of any sperm consists chiefly of the nucleus, whose subsequent development involves principally a change of shape and a concentration of the chromatin. In the present case the former process consists of a very considerable elongation, but the alteration is in no way exceptional and requires no further comment. The structures of primary interest, therefore, are the acrosome, middle piece, and tail, whose histories it will be convenient to treat separately.

Considering first the origin of the acrosome, or tip of the head, we find it to be formed from the larger part of the idiozome in the following manner: This part of the idiozome assumes the form of a vacuole within which and against one side there lies a darkly staining body. The remainder of the idiozome partially surrounds this vacuole in the form of fine granules (*B, C*). The entire mass now moves until the side of the vacuole containing the dark body has come in contact with the nucleus (*D*). The granular remains of the idiozome then become separated from the major vacuolated portion, while the cytoplasm shifts its position in such a manner that the latter portion protrudes from the surface of the cell (*E*). Upon its inner
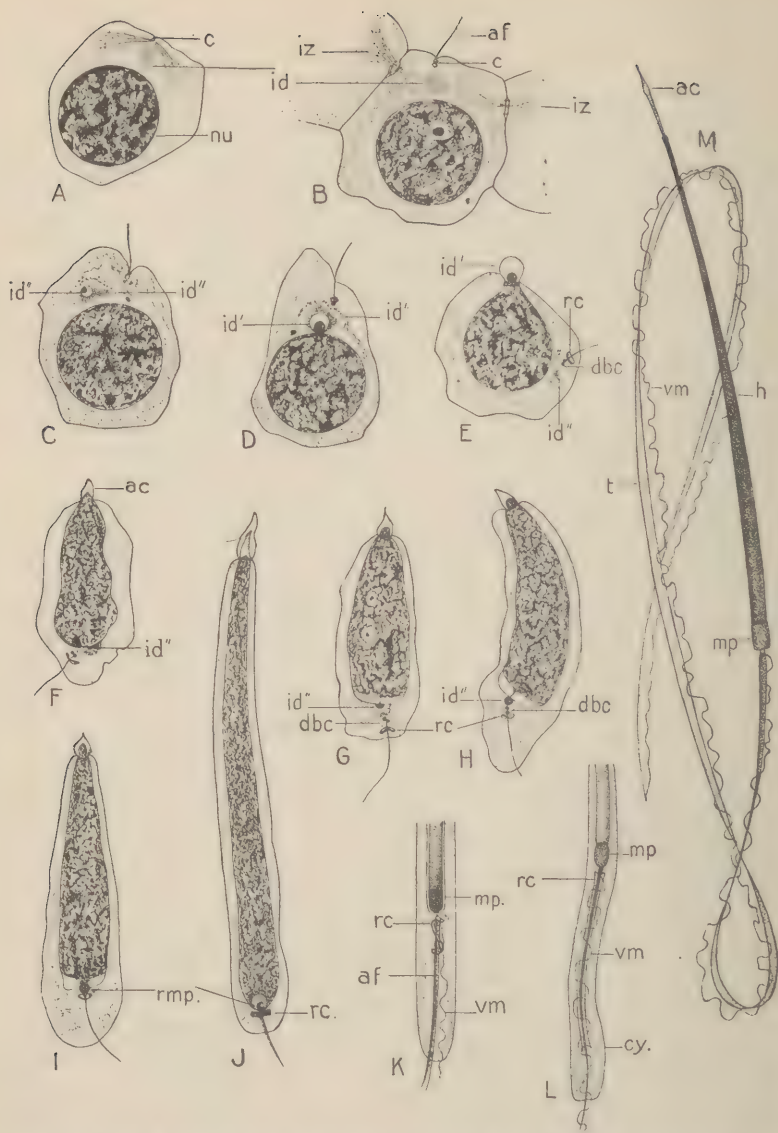
Fig. 17. — After McGregor. See text for explanation of stages. *ac.* Acrosome derived from vacuolated part of idiozome. *af.* Axial filament of the tail derived from the centrosomes. *c.* Centrosome. *cy.* Cytoplasmic envelope covering the head and growing over the anterior part of the tail. *dbc.* Dumb-bell shaped centrosome or end knob, which forms part of the middle piece. *h.* Head of mature spermatozoan. *id.* Idiozome. *id'.* The vacuolated portion of the idiozome which gives rise to the acrosome. *id".* The granular portion of the idiozome which, with the dumb-bell shaped centrosome, gives rise to the middle piece. *iz.* Remains of the inter onal fibers from a previous cell division passing through a ring shaped mid-body. *mp* Middle piece. *nu.* Nucleus which forms the main part of the head of the spermatozoan. *rc.* Ring centrosome. *rmp.* Rudimentary middle piece in process of formation. *t.* Tail of mature spermatozoan. *vm.* Vibratile membrane.

side, however, at the point containing the darkly staining body, this vacuolated part of the idiozome still remains attached to the nucleus. Its form then gradually changes along with that of the nucleus itself, so that it finally becomes the sharp acrosomal extremity of the elongated head.

Regarding secondly the origin of the middle piece and tail, it appears that their development is dependent chiefly upon the centrosome and the granular remnant of the idiozome. At the beginning of the processes already described the centrosome becomes incompletely divided into two. One of these lies at this time against the cell wall, which is often slightly depressed to meet it, and from it there grows outward a fine thread, the axial filament of the tail. The other centrosome is toward the interior of the cell and thus nearer the nucleus (*A, B, C*). The more peripheral centrosome now gradually assumes a ring form, while the more central one becomes first spherical and then dumb-bell shaped (*D, E*). Also the latter presently separates somewhat from its ring-shaped mate, though remaining connected with it by a delicate strand. Furthermore, because of the fact that this strand appears to be an actual extension of the axial filament, possessing the dumb-bell shaped centrosome for its inner termination, the latter is often referred to as the *end knob* (*E, F*). The major part of the idiozome has meanwhile become separated from the smaller granular portion in the manner previously indicated. Also accompanying the above mentioned shifting of the cytoplasm, the granular part of the idiozome, together with the centrosomes, has migrated to what proves to be the posterior or tail side of the sperm head (*D, E, F*). This granular idiozomal remnant next fastens itself to the nucleus in this region, and assumes the form of a darkly staining globular body (*F*). From it a fine thread enters the base of the nucleus and traverses the clear space which appears there at this time (*G, H*). The globular body then passes into the nucleus, and in the process carries the end knob (i.e. the dumb-bell shaped centrosome) with it (*I, J*). The two elements have meanwhile become indistinguishable from one another and together form the middle piece of the spermatozoan (*K*). While this has been going on the axial filament has continued to grow out, and has given rise along its border to a delicate vibratile membrane. The ring centrosome then becomes greatly elongated, one end of it lying next to the base of the middle piece and the other some distance down the tail
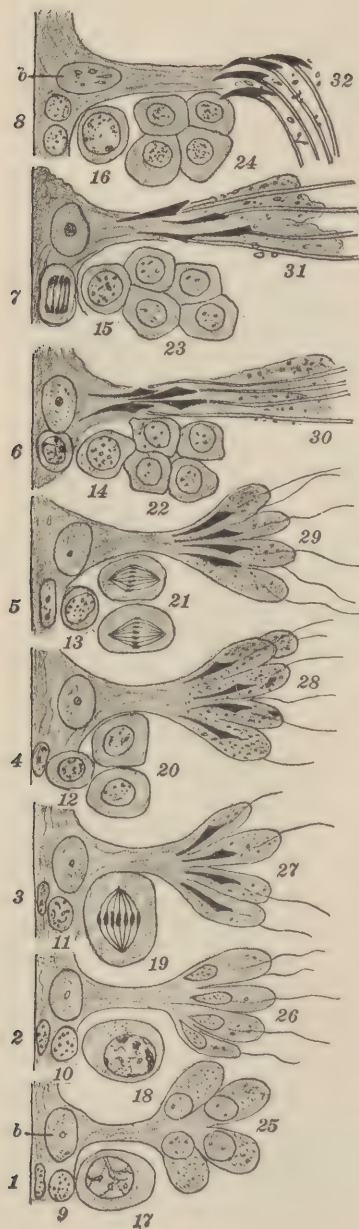
(K, L). Apparently the entire ring ultimately becomes fused with the axial filament. As these changes occur the cytoplasm elongates and finally forms a very thin envelope for the head and one side of the anterior portion of the tail. Thus the spermatid assumes the shape of the mature spermatozoan (M).

The entire development may now be summarized as follows: The nucleus forms the main part of the head, and the vacuolated portion of the centrosphere produces the acrosome. The granular remnant of the centrosphere and part of the centrosome develops into the middle piece, the remainder of the centrosome giving rise to the axial filament of the tail. Part of the latter, as well as the head, is covered by a thin envelope of cytoplasm.

Further Differences between the Development of the Sperm and the Ovum. — Having completed the description of the metamorphosis of

Fig. 18. — Diagrammatic outline of the spermatogenesis of the Rat in thirty-two stages. From Kellicott (General Embryology). After v. Ebner. Theca of tubule toward the left. Lumen of the seminiferous tubule toward the right.

1–8. Period of multiplication (the number of cell generations is actually very large). 9–18. Period of growth. 19–24. Period of maturation. 25–32. Period of metamorphosis. b. Basal cells or Sertoli cells. 1–16. Spermatogonia. 17, 18. Primary spermatocytes preparing for division. 19. First spermatocyte division. 20. Secondary spermatocytes. 21. Secondary spermatocyte division. 22–25. Spermatids. 26–31. Transformation of spermatids. 32. Fully formed spermatozoa.

the sperm we may now pause to emphasize two further differences between the development of the latter and that of the ovum. The first is the fact that the multiplication of spermatogonia does not cease during the sexual life of the animal. Hence it is more true here than with the ovum that only the early generations, or part of them, arise from the primordial germ cells which have migrated from the gut. Instead the majority are apparently continually being derived from the indifferent epithelial cells of the seminiferous tubules. For this reason all of the above stages of development are always to be found in these tubules. Where there are no cysts, the youngest cells occur next to the epithelium and the older ones successively nearer the central lumen. Where there are cysts, on the other hand, any one, at a given time, usually contains only cells of one stage.

Another difference which may be noted is the arrangement of the developing sperm relative to their source of nutriment. It has already been indicated that the cells (*Sertoli cells*) which furnish this do not, except sometimes in the earliest stages, surround each spermatozoön. Instead they form the lining to either a tubule or cyst containing many such germ cells. Then as the development of these cells proceeds, they become arranged in bundles, all the heads of one bundle becoming imbedded in a single nutrient cell. When the sperm are mature the cyst wall, if there be one, breaks so that their tails project freely into the lumen of the tubule. At the same time the spermatozoa becomes loosened from the Sertoli cells and are thus ready to be released into the above mentioned lumen (Fig. 18).

MATURATION

It is now necessary to return to the consideration of a process which is common to both ovum and sperm; i.e., *maturation*. As has already been indicated, the phenomenon is a rather complicated one. Furthermore, it varies somewhat in different animals, and the exact meanings of some of its stages are still in considerable doubt. For the sake of necessary brevity and clearness, therefore, it will be necessary to limit rather sharply the varieties described, and the possible interpretations of which their stages are susceptible.[4] Also, inasmuch as there

[4] For a full discussion of this subject with references to the complete literature the student is referred to *The Cell in Development and Inheritance* by E. B. Wilson.

are differences in the behavior of the ovum and sperm, it will be necessary to describe them separately. The male germ cell will be considered first.

The Maturation of the Spermatocyte. —

I. The Lepotene Stage. — Shortly after the last spermatogonial division, the chromatin of the enlarging nucleus arranges itself in a *spireme*, or as it is sometimes called, a *leptotene thread* (Fig. 19, 2, 3). This spireme, according to the interpretation here adopted, is similar to that which occurs in the division of an ordinary cell and it presumably consists, therefore, of the chromosomes adhering together end to end (Fig. 20, I).

II. The Synaptene Stage. — In this stage the spireme thread appears double, and frequently becomes arranged in portions which seem to converge toward the side of the nucleus nearest the centrosome (Fig. 19, 4). In many instances it seems that each of these portions is really a very much elongated loop, the close approximation of whose sides gives to the portions their double aspect. These features it should be noted are among those susceptible of a variety of interpretations. Continuing upon the basis of the one here adopted, however, the constitution of each double portion of thread is as follows: Each side of such a portion, or loop, if one wishes to regard it as such, represents one member of a chromosomal pair. This then simply means that in every pair the two members composing it have approached one another closely throughout their length (Fig. 20, II). If this is indeed the explanation the number of the double portions of spireme should be just half the somatic number of chromosomes. Unfortunately, however, the threads in this stage are so fine and tangled that they give only the general impression described above, and it is impossible to determine at all accurately how many there are.

III. The Contraction or Synizesis Stage. — The thin double appearing threads now begin to thicken somewhat. At the same time they are often drawn into a closely tangled mass, a condition referred to as *contraction* or *synizesis* (Fig. 19, 5; Fig. 20, III). This phase frequently persists during almost the entire growth period, though in some cases it is the fifth stage (diplotene) which lasts longest.

IV. The Pachytene Stage. — It should be noted that this fourth condition may precede that of contraction instead of following it. Adhering to the order here indicated, however, as the thickened threads emerge from synizesis, the significant fact becomes evident that they

are no longer double (Fig. 19, 6, 7; Fig. 20, IV). Furthermore, since
they are now relatively shorter and straighter, it is sometimes
possible to estimate fairly accurately their number, which is thus
found to be about half that of the number of chromosomes in a
somatic cell. If then the latter is termed the *diploid* number and
designated by s, the reduced number may be termed *haploid* and
designated by $\frac{s}{2}$. It will now be recalled, however, that such a haploid
number was exactly what was postulated with respect to the pairs of
laterally opposed threads in the synaptene. It will also be remem-
bered that each of these pairs of threads was assumed to represent
actually a pair of chromosomes. What has now apparently taken
place, therefore, is the lateral fusion of the two members of each
chromosomal pair, thus producing in each instance a thread which ap-
pears thicker but single. Such a side by side union as this is termed
parasynapsis, as opposed to only an end to end union or *telosynapsis*,
which is said to be all that occurs in certain forms. No one knows
just how complete this parasynaptic union is. There is some evidence,
however, that it involves the twisting about each other of the two mem-
bers of a pair, and that before they separate a certain amount of
material may be exchanged.

V. The Diplotene Stage. — Following the pachytene stage the
chromatin threads no longer converge toward the idiozome, and again
appear double (Fig. 19, 8, 9). Counting each section of double thread
as one, however, their number is still distinctly haploid. The question
at once arises: is this double appearance to be interpreted in the same
way as was the double aspect in the synaptene? According to one view,
it is, in which case the double threads again represent members of
chromosomal pairs lying side by side (Fig. 20, V, a). According to
another view the paired chromosomal threads are still fused. The
new split which has appeared in them therefore does not represent
the space between two chromosomes of an original pair. Instead it
represents a splitting which has occurred along the longitudinal axis
of each thread, at right angles to the plane of their fusion (Fig. 20,
V, b). As will appear presently the interpretation adopted determines
also the interpretation of certain events which follow. It does not,
however, modify our conception of the final results.

A second contraction or synizesis stage may now ensue, during which
the threads are drawn toward the center of the nucleus and their
identity becomes indistinct for a time.

VI. The Diakinesis Stage. — Eventually the double threads re-appear in haploid number and shorten to form chromosomes. These chromosomes, however, are not in the form of simple rods or spheres, as in the case of normal cell division. Instead they are usually in the
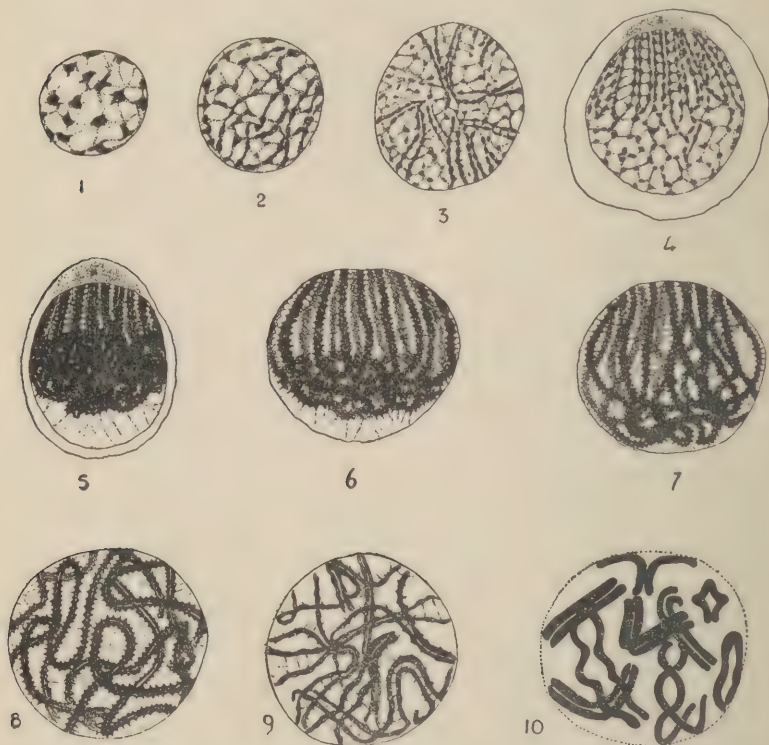
Fig. 19. — Prophases of the heterotype division in the male Axolotl. From Jenkinson (Vertebrate Embryology).

1. Nucleus of spermatogonium or young spermatocyte. 2. Early leptotene. 3. Transition to synaptene. 4. Synaptene with the double filaments converging towards the centrosome. 5. Contraction figure. 6, 7. Pachytene. 8. Early. 9. Later diplotene. 10. The heterotypic double chromosomes; the nuclear membrane is disappearing.

shape of crosses, rings, loops, and other curious figures; i.e., *hetero-typic* (Fig. 19, 10). The reason for this is presumably their peculiar composition, for it must be remembered that, whichever of the above views of the diplotene be adopted, each mass of chromatin now present represents in some form what was originally two chromosomes and is about to become four (Fig. 20, VI, a, or VI, b).

VII. The First Maturation Division. — The above chromatin figures are presently arranged at the equator of an ordinary amphiaster, but because of the peculiar aspect of these figures the division which ensues is often termed *heterotypical* (Fig. 21, 1, 2, 3; Fig. 22, I, a, or I, b). It appears that during this division the chromatin is probably separated along the line of the split in the haploid threads of the

Fig. 20. — A generalized diagram of the earlier processes of maturation leading up to the maturation divisions. For the sake of simplicity only six chromosomes (three pairs) are figured, and the extra nuclear portions of the cells are omitted. The members of a single pair bear conventional markings similar in form but distinguishable by the fact that those of one member of a pair are darker than those of the other.

I. The *leptotene stage*, showing the thread-like chromosomes strung together end to end. II. The *synaptene stage*, showing the two members of each pair lying side by side, all the pairs apparently attached by their ends to the nuclear membrane. III. The *contraction stage (synizesis)*, showing the paired threads coiling up. In this figure and those following the members of the stippled pair at the left of the nucleus are represented as twisting about one another. It is practically certain that this twisting process occurs in some cases of parasynapsis (see text) but perhaps not in all. Hence the other two pairs are not so figured. IV. The *pachytene stage*, showing the complete side to side (parasynaptic) fusion of the members of each pair. V.a. The *diplotene stage*, assuming that the members of the pairs have slightly separated from one another along the original line of fusion (reduction). V.b. The *diplotene stage*, assuming that the renewed double appearance of the threads is due to an equational split in the fused members of each pair. VI.a. The *diakenesis stage*, or prophase of the first maturation division, assuming that this division is to be reductional. VI.b. The *diakenesis stage*, assuming that first maturation division is to be equational. Tetrad formation is slightly suggested in stage VI (see text).

diplotene. In some cases, indeed, this is fairly clear, but in instances where a second contraction occurs the situation is somewhat obscured.

Granting, however, that the line of division is as indicated, our interpretation of its significance depends chiefly upon which view

is held as to the nature of the diplotene split. If this split represented the separation between a pair of chromosomes which had fused side by side during the pachytene, then the first division necessarily means the drawing apart of those same chromosomes. In that event each of the new cells receives one complete $\frac{s}{2}$ set of the whole chromosomes and the division is therefore said to be *reductional* (Fig. 22, I, a; Fig. 23, A, I, II). If on the other hand the split was simply the longitudinal division of each chromosome of a fused pair, then, aside from the fact that the chromosomes have been split while fused, the

Fig. 21. — First maturation division in the male. 2. Salamander, the remainder Axolotl. From Jenkinson (Vertebrate Embryology). 1, 2. The heterotypic chromosomes on the spindle (metaphase). 3. Anaphase. 4, 5. Telophase. 6. Resting nuclei. 4–6. Cell-division into two secondary spermatocytes.

operation is not different from ordinary mitosis. In that case each cell receives not one complete set, but a regular double set, the members of whose pairs are, however, united with one another. In such an event there has been no true reduction and the division is said to be *equational*. It is important to note, however, that even in the latter event the number of chromosomes appears haploid because of the fusion. Also, the contour of the fused halves of a pair is likely to look about the same as one whole member of that pair (Fig. 22, I, b; Fig. 23, B, I, II).

VIII. The Second Maturation Division. — Until the completion of the first division, the spermatocyte is known as *primary*. After that it is called *secondary*. The secondary spermatocyte generally enters upon a brief period of rest preceding the next division (Fig. 21, 4, 5, 6). During this time the nucleus is often reconstituted, and the chromatin assumes to varying degrees the typical resting condition. Presently, however, the chromosomes emerge from this stage in the usual manner and become arranged on the spindle preparatory to the second division. On this occasion they generally present a normal appearance, aside from the important fact that their number
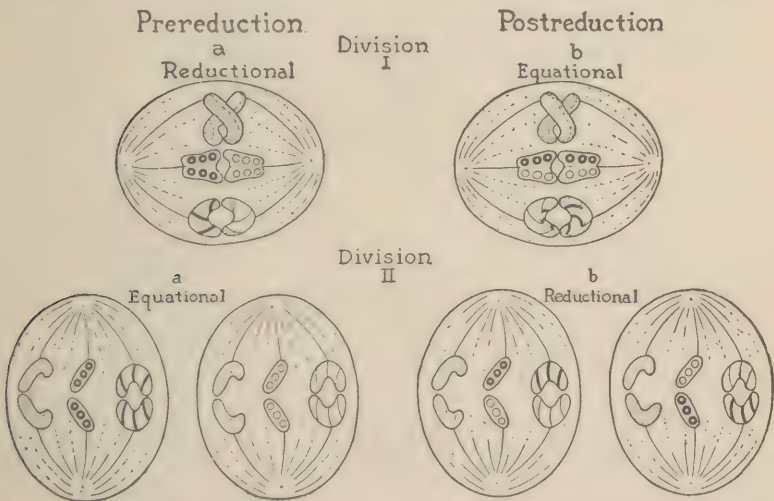
Fig. 22. — A continuation of the diagram in Fig. 20 showing the possible arrangements of the chromosomes in the first and second maturation divisions. In each instance the division is represented at the metaphase.

Pre-reduction. Division I.a. The first maturation division, in this case reductional and therefore to follow VI.a. in Fig. 20. Division II.a. The second maturation division undergone by the two products of the first division in the case where that was reductional. This second one is therefore equational. *Post-reduction.* Division I.b. The first maturation division but in this case equational and therefore to follow VI.b. in Fig. 20. Division II.b. The second maturation division undergone by the two products of the first when that was equational. This second one is therefore reductional. Note that so far as the matter of pre-reduction or post-reduction is concerned the ultimate chromosomal distribution in the four cells finally produced is the same in either case. It should be added, that no significance attaches to the fact that in both reductional divisions in this figure all of the darkly marked chromosomes are represented as always going together to one pole, and all the lighter ones to the other. This is merely for the sake of simplification and clearness. As a matter of fact the members of any given chromosomal pair in these divisions behave quite independently of the members of other pairs as regards the pole to which they happen to move.
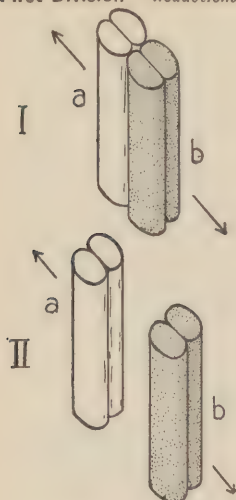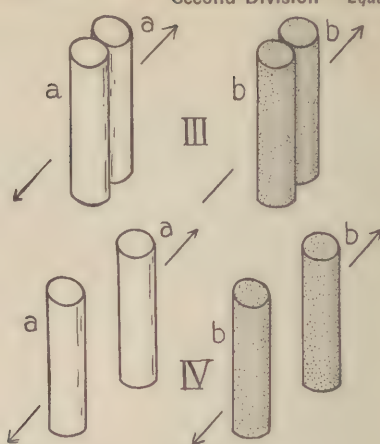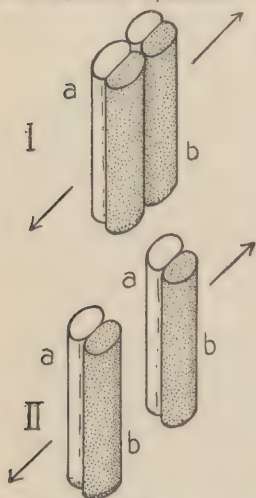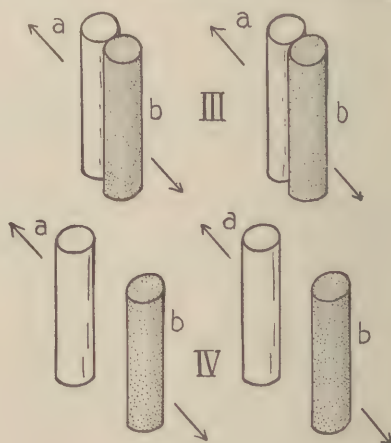
Fig. 23. — A stereoscopic diagram representing the two possible types of behavior of one of the three pairs of chromosomes indicated in Fig. 22 during the first and second maturation divisions. The letter *a* designates one member of the pair and *b* the other member. For the sake of clearness the plane of the second division is indicated in both types before the first division has actually started. This suggests the condition in tetrad formation (see text). It does not really represent that condition, however, because in "genuine" tetrads as strictly defined the four bodies are entirely separate at this stage.

In the upper set of four figures the first division (that on the left side) is reductional, i.e., *a* and *b* are separated from one another, while the second division (that on the right side) is equational, i.e., *a* and *b* are each split in half (*Pre-reduction*). In the lower set, on the other hand, the first division (that on the left side) is equational, i.e., *a* and *b* are each split in half, but in each instance the half of *a* remains attached to the half of *b*. The second division (that on the right side) then follows and in each half which resulted from the first division the *a* portion is separated from the *b* portion (*Post-reducrion*).

remains haploid (Fig. 22, II, a, or b). A regular mitotic division now follows, and each cell again receives its $\frac{s}{2}$ quota of chromosomes. Because of the essentially normal appearance of this division it is termed *homotypical*.
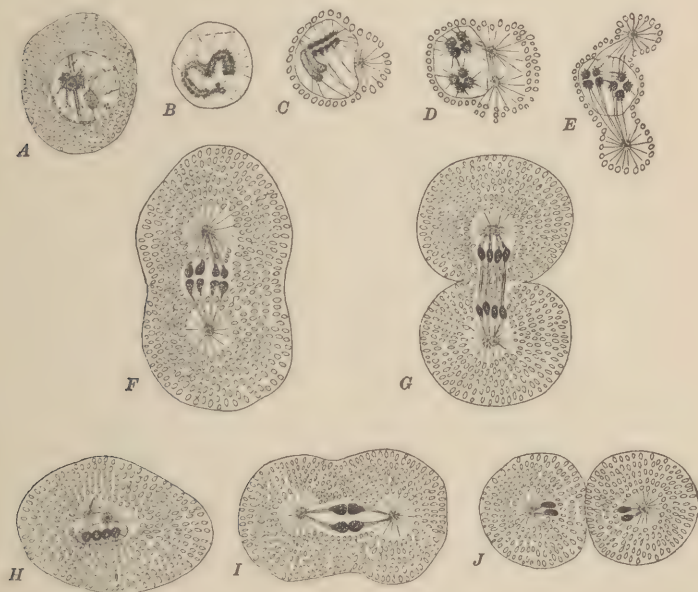
Fig. 24. — Tetrad formation in the spermatogenesis of *Ascaris megalocephala bivalens*. From Kellicott (General Embryology). After Brauer. x 795. A-G. Stages in the division of the primary spermatocyte. A, B. Splitting, and C, condensation of chromatin thread, seen in side view. D. shows, in end view, that the splitting is double. Centrosome divided. E. Migration of centrosomes and formation of spindle. F, G. Division of the cell body and of the two tetrads. H. Secondary spermatocyte containing two dyads. I. Division of secondary spermatocyte. J. Two of the spermatids, each with two " monads " or single, univalent, chromosomes.

The genuine nature of this division, however, again depends upon our interpretation of the diplotene, and the consequent character of the first division. If the split in the diplotene was such that the first division was reductional, then the second division must be equational (Fig. 22, II, a; Fig. 23, A, III, IV). If on the other hand the condition of the diplotene was such that the first division was equational, then the second division is probably reductional (Fig. 22, II, b; Fig. 23, B, III, IV). If the former procedure is thought to hold, the process is termed *pre-reduction* (Fig. 22, I, II, a; Fig. 23, A); if the

latter obtains, it is *post-reduction* (Fig. 22, I, II, b; Fig. 23, B). It should be noted, however, that it is always difficult and frequently impossible to be sure which type of maturation has occurred. The reason for this is three-fold: first, there is the difficulty of interpreting the nature of the diplotene; second, although this be
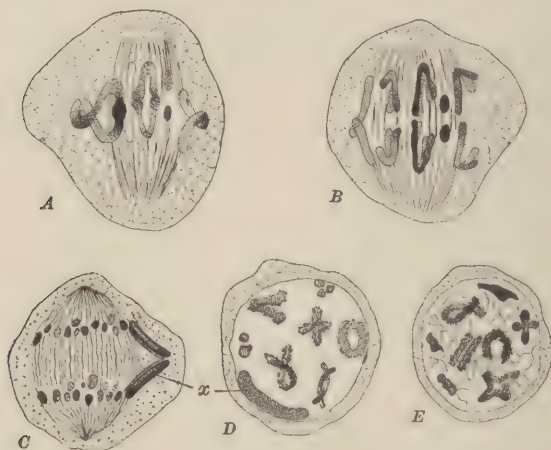
Fig. 25. — Maturation divisions in certain Insects, showing forms of chromosomes and their relation to tetrads. From Kellicott (General Embryology). After de Sinéty. x 1125. A, B. Two stages in anaphase of primary spermatocyte division in *Stenobothrus parallelus*. Rings opening into Vs which diverge. C. Anaphase of spermatogonial division in *Orphania denticauda,* showing differentiated chromosome, x. D, E. Preparation for first spermatocyte division in *Orphania*, showing " tetrads " in various stages of formation from rings and crosses, i.e., diakinesis figures.

fairly certain there is often a second contraction stage to obscure the relation between the diplotene and the first division, and thirdly, as noted above, the chromosomes which result from this division are similar in number and appearance, whether reduction has taken place or not. However, whether reduction occurs in the first division or the second, it is now clear, as indicated, that the final result is the same; that is, there are produced four spermatids, each containing one haploid set of chromosomes.

This last statement, it should be added, is frequently not precisely true. The exception is exceedingly important, but it has been omitted for the time being for the sake of clearness. It can be better appreciated, furthermore, when described in connection with the con-

dition in the ovum. We shall reserve this point, therefore, until after the description of maturation in the female.

Maturation by Tetrad Formation. — The earliest cases of maturation described were those in certain Invertebrates in which the process, as indicated above, was varied in the following manner: Following the diplotene stage, or something equivalent to it, the chromosomes appear as usual in haploid number. Each of these condensed masses of chromatin is peculiar, however, in that it does not consist of a cross or other shape which is merely *suggestive* of a double or even quadripartite constitution; instead when properly viewed each chromosomal mass is seen to be composed definitely of four *clearly separated parts,* for which reason it is termed a *tetrad.* The first spermatocyte division then occurs, after which there continue to appear in each of the resultant cells the haploid number of chromatin aggregations; each aggregation now consists, however, of only two parts, and is therefore called a *dyad.* Immediately following this, without the usual intervention of a semi-resting stage, there ensues the second spermatocyte division, resulting in the formation of the spermatids, and each spermatid contains the haploid number of single chromosomes. A brief consideration of this phenomenon will make clear that it is essentially similar to the more common method of maturation, both in the events which take place and in the possible interpretations to be put upon them. This becomes evident from an analysis of the chief points distinguishing the more eccentric method, which are readily resolved into two. The first and more fundamental consists in the fact that the split in the chromosomes preparatory to the second division takes place before the first division has occurred, thus giving rise to the four part chromatin aggregations or tetrads. The other point; i.e., the absence of any appreciable pause between the two divisions, is then obviously correlated with the first. That is, the early occurrence of the split preparatory for the second division apparently makes unnecessary any extended intermitotic interval (Fig. 24).

The entire process thus described is known as maturation by tetrad formation, and because of its early discovery and its clearness in some instances it has received considerable emphasis. Later investigation, however, has tended to show that its occurrence in exactly the manner indicated above is relatively rare, the best known and clearest cases, perhaps, being those among certain of the Nematodes. Nevertheless, it should be added that such clear-cut instances are con-

Fig. 26. — From Kellicott (General Embryology).   A. Chromatin extrusion from the nucleus into the cytoplasm in the oöcyte of the Medusa, *Pelagia noctiluca.* After Schaxel.   B. Extrusion of chromatin into the cytoplasm during the maturation of the oöcyte of *Proteus anguineus.* After Jörgensen.   x 1080.

nected by many gradations with those in which any suggestion of true tetrad formation as strictly defined above is entirely absent. Such transitional stages indeed are particularly common among the Arthropods, and serve still further to emphasize the fact that in their essential features the two types of maturation are probably identical (Fig. 25).
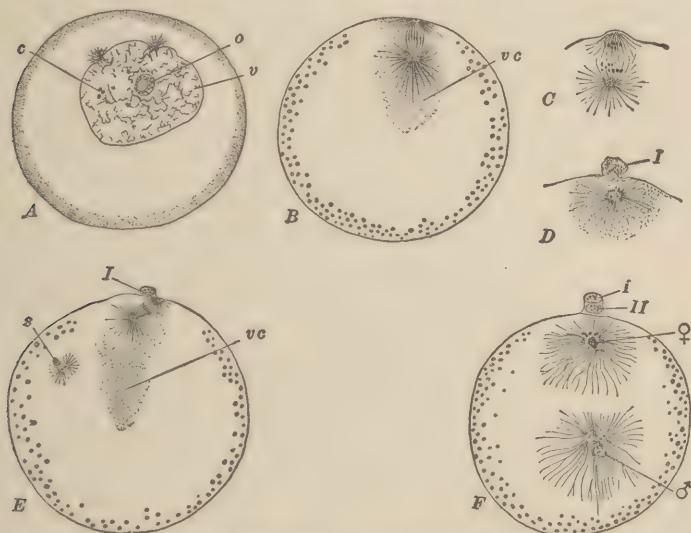
Fig. 26I. — Maturation and fertilization in the Nemertean, *Cerebratulus*. From Kellicott (General Embryology). After Coe. C, D, x 375, others x 250. A. Primary oöcyte. Part of the chromatin has been condensed into chromosomes, only five of which are shown (the number present is sixteen). The remainder of the chromatin is thrown out into the cytoplasm. The centrosomes, each with a small aster, are diverging, and the nuclear membrane is commencing to disappear. B. First polar spindle fully formed and rotated into radial position. Chromosomes in equatorial plate. The extra chromatin (*vc*) is seen scattering through the cytoplasm. C. First oöcyte division; anaphase. D. First polar body nearly separated. E. First polar body completely cut off; second polar spindle formed and rotating into radial position. Spermatozoön within the egg. F. Second polar body completely separated. Egg pronucleus forming, surrounded by large aster. Sperm pronucleus, also with a large aster, enlarged and approaching the egg pronucleus. These steps connected with the behavior of the egg and sperm nuclei (pronuclei) will be fully explained later on in the text.

c. Chromosomes. *o*. Nucleolus, vacuolated and commencing to disappear. *s*. Spermatozoön just within the egg. *v*. Germinal vesicle. *vc*. Extra chromosomal chromatin beingscattered through the cytoplasm. I, II, First and second polar bodies. ♂ Sperm nucleus (pronucleus). ♀ Egg nucleus (pronucleus).

The Maturation of the Ovum. — Maturation in the ovum is fundamentally similar to that in the sperm, with certain variations in detail. It will be possible, therefore, to make clear the process in the oöcyte by simply indicating the points in which it differs from that just described. These points may be stated as follows:

I. Length of Early Stages. — In some instances at least, the early maturation stages up to and including synizesis occur immediately after the last oögonial division. In any event, as previously noted, this stage for all ova is usually reached at the time of hatching or birth of the female containing them. Furthermore, contrary to the case of the sperm, no more ova are produced, and hence some of them must
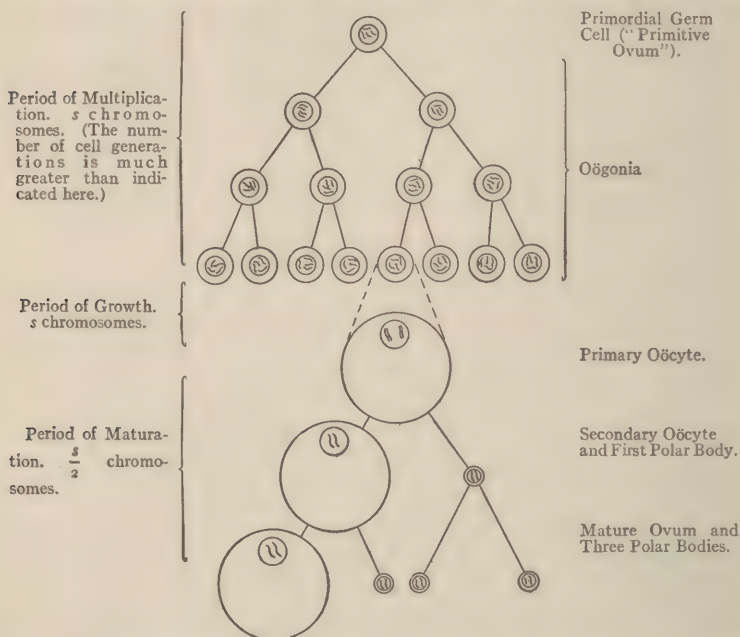
Fig. 27. — Diagram of the chief events of oögenesis. Modified from Kellicott after Boveri. Compare with Fig. 28.

remain in this stage throughout the sexual life of the individual. Thus in the human female, synizesis in the last ovum to mature must last for forty or fifty years before the threads finally emerge in the diplotene. In the sperm, on the other hand, the development of any particular cell never continues for any such extended period.

II. Loss of Chromatin. — From the theoretical point of view, one rather notable occurrence in the ovum during its maturation is the loss of chromatin into the cytoplasm. This loss may occur during the growth period, but more frequently takes place at its close when the chromosomes are forming for the first division. It may then be accomplished by the breaking up of parts of the diplotene threads,

whose fragments are cast out of the nucleus. In other cases, the threads which re-form after synizesis do not use all of the chromatin and the remainder is thrust into the cytoplasm (Figs. 26, 26I). Such a loss of chromatin as this rarely occurs in the case of the sperm.

III. Size of the Division Products. — Perhaps the most striking of all the differences between maturation in the ovum and that in the sperm is the difference in the size and fate of the products of the two divisions. In the sperm, as has been noted, the two maturation

Period of Multiplication. s chromosomes. The number of cell generations much greater than is indicated here.

Period of Growth. s chromosomes.

Period of Maturation. $\frac{s}{2}$ chromosomes.

Period of Metamorphosis. $\frac{s}{2}$ chromosomes.

Primordial Germ Cell.

Spermatogonia.

Primary Spermatocyte.

Secondary Spermatocytes.

Spermatids.
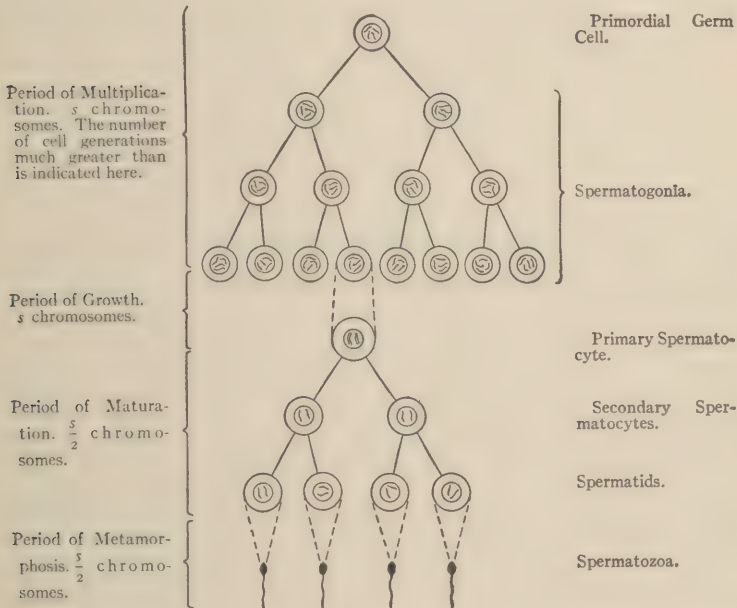
Spermatozoa.

Fig. 28. — Diagram of the chief events of spermatogenesis. Modified from Kellicott after Boveri. Compare with Fig. 27.

divisions are equal and the resulting four cells are all alike and functional. In the ovum, on the other hand, the cytoplasmic divisions in both cases are extremely unequal and only one of the four final products is a functional egg cell. The others are relatively minute and are known as *polar bodies*, the one resulting from the first division being termed the first polar body and that resulting from the second division the second polar body. This condition of inequality is brought

about by the fact that at each division the nucleus and division mechanism take up a position at the periphery of the cell instead of at its center. Thus one set of chromosomes remains in the main cell, while the other set is pinched off in a very small bit of cytoplasm (Fig. 26I).

Although there is this great discrepancy in the distribution of the cytoplasm, there is good reason to believe that the nuclear content is the same in every case, just as it is in the sperm. In other words, the performance is in every way homologous with the two spermatocyte divisions except for the inequality in the distribution of the cytoplasm. This idea is borne out by the fact that in many cases, as might be expected, the first polar body divides again as does its larger sister cell, thus producing one ovum and three polar bodies. This behavior in the case of the ovum is thought to be an adaptation to secure the greatest amount of cytoplasm and nutriment in a single cell.

IV. The Time of the Maturation Divisions. — In the sperm, as has been seen, maturation is entirely completed within the testis and before the spermatid even enters upon its final period of development. In the ovum, on the contrary, maturation, at least so far as the divisions are concerned, is the last thing to occur. Sometimes division takes place while the ovum is in the ovary. More frequently, however, especially among the Vertebrates, at least one of the two divisions occurs after the ovum has left the gonad. Indeed in many cases the second division does not take place until after the egg has been entered by a spermatozoön (Fig. 26I). A comparison of the chief processes involved in the development of the sperm and ovum is presented diagrammatically in Figs. 27 and 28.

The Sex Chromosomes. — We are now prepared to return to a consideration of the exception in chromosomal behavior which was noted but not described at the end of the account of maturation in the sperm (p. 46).

In the description of the typical cell there was presented some evidence to prove that chromosomes are qualitatively different, and this point has received further emphasis from a study of the germ cells. In order to avoid confusion, however, it has thus far been assumed that, though the chromosomes may differ in quality, their behavior is fairly uniform. In general this is true, but it is now necessary to note an important instance where it is not.

In the somatic and germ cells of many animals, both male and female, there are found one or more chromosomes which in many cases behave quite differently from their fellows. They often stain more deeply, and are especially peculiar in that they frequently remain in the condensed condition during the entire growth period of the germ cells. On this account they sometimes appear at this time like nucleoli with various distinctive shapes (Fig. 29). Also, during the anaphase stage of cell division, they are noted for a tendency to lag behind on the spindle (Fig. 30). One of the most striking things
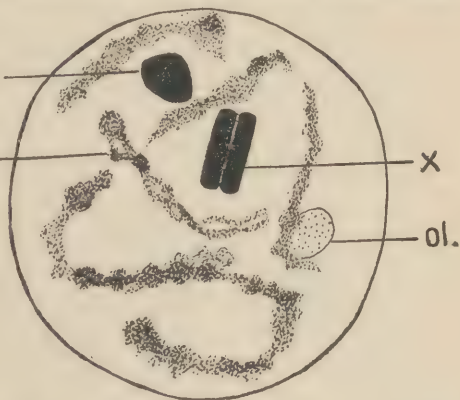
Fig. 29. — A diplotene nucleus in *Lygaeus bicrucis*. After E. B. Wilson. Note the condensed condition of the sex chromosomes, X and Y. The remaining chromosomes, on the other hand, are still thread-like, one of them, *a*, as well as the sex chromosome X, showing the characteristic diplotene split. This split in the case of the X is obviously equational. The plasmosome, *pl*, is only partly visible.

about these chromosomes, however, is the fact that in some animals in the male, each somatic cell, as well as each unmaturated germ cell, possesses only one of them, while each cell of a similar type in the female has two. Under such conditions the one or two eccentrically behaving chromosomes are termed *X chromosomes*. In such cases it follows of course that in the male the total number of chromosomes in each cell of the types indicated is odd, whereas in the female the number in each cell of a similar type is even. Thus, taking the insect *Protenor* as an example, such cells in the male each possess 13 chromosomes and those in the female 14. Under such circumstances it is obvious that when the male germ cell comes to maturate, its X chromosome will be without a mate. Apparently as a result of this fact the odd chromosome in the male only divides at one of the maturation divisions; e.g., in the instance in question the first, and since this chromosome has not had a mate its division must presumably be equational (Fig. 31, *C*). Following the second division, the final result, as usual, is four male germ cells, but their content is obviously not quite equal. Two of them possess six ordinary chromosomes (*autosomes*), while each of the other

two possesses a similar six autosomes, and in addition an X chromo-
some; i.e., a total of seven (Fig. 31, *D, E, D¹, E¹*).

It has already been noted that the female in such cases as that of
the example cited, has an even number of chromosomes in her somatic
cells (e.g., in this instance 14), and that two of them are of the X
type. As has also been indicated, these two chromosomes in some
animals do not enter a spireme condition during the maturation of the
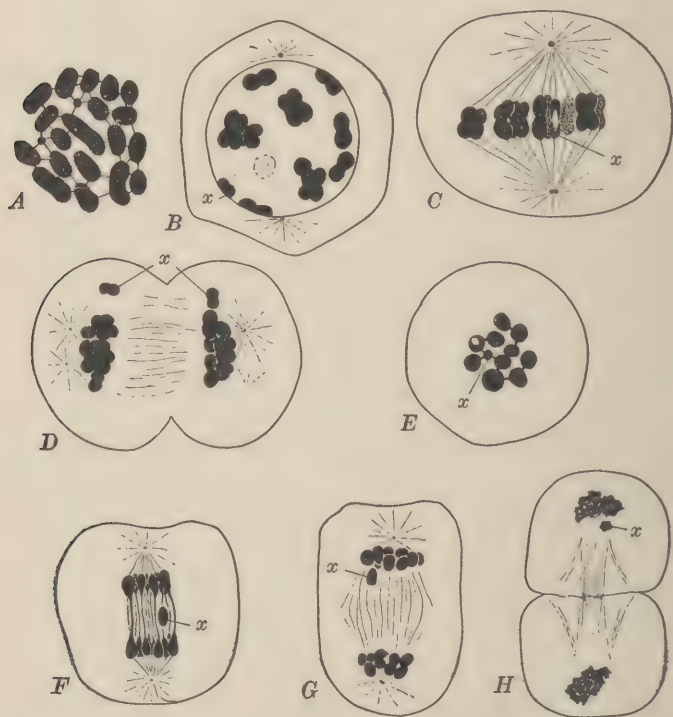
Fig. 30. — Maturation during the spermatogenesis of the squash-bug, *Anasa tristis*,
showing the behavior of the X-chromosome or idiochromosome.   From Kellicott
(General Embryology). A, After Wilson, others after Paulmier. A. Spermatogonium.
Polar view of equatorial plate showing twenty-one chromosomes (ten pairs, plus one).
The X-chromosome is not distinguishable at this time. B. Primary spermatocyte.
Tetrads formed. C. Equatorial plate of first spermatocyte division. X-chromosome
divided. D. Anaphase of same division. The daughter X-chromosomes have also
diverged.   E. Equatorial plate of second spermatocyte division.   F. Anaphase of same
division. The X-chromosome lies, undivided, between the two groups of daughter
chromosomes. G. Late anaphase of same division. The undivided X-chromosome
has passed to the upper pole, lagging behind the others. H. Telophase of same
division. X-chromosome still distinct.

germ cells, and therefore cannot mate with each other in quite the same way that the others do during synapsis. In most cases, however, even though these X chromosomes do not assume the thread-like condition, they do fuse with one another at some time previous to division. This union may occur quite early, and may last for some time, while in other cases it is very brief and occurs just previous to the arrangement of the chromosomes on the spindle. In the two maturation divisions which follow, the single body formed by the fusion of the two X chromosomes divides each time. The assumption, there-fore, that in the case of this chromosomal pair one of these divisions is truly reductional, is very strong. Indeed, in some instances the fusion is so slight, and the arrangement of the double body on the spindle so clear, that it is almost certainly so. The result of these divisions of the oöcyte is, for reasons already explained, only one functional egg. The significant point, however, is the fact that this egg always contains half of some even number of chromosomes; e.g., in the case we have been citing, seven (Fig. 32).

To appreciate the full significance of the phenomena just described, it is now necessary to anticipate slightly the subject of fertilization. From what has been said it is evident that, in the type of chromo-somal arrangement thus far considered, the sperm are of two kinds, half of them containing the haploid number of autosomes only, and the other half this same number of autosomes plus one X. The eggs on the other hand will obviously be all alike, each one containing both the X and the haploid number of autosomes. Thus in the case of *Protenor*, as previously indicated, the total number of chromosomes in each type of sperm is respectively 6 and 7, whereas every egg con-tains 7. Continuing to take this animal as an example, it is then clear that after random mating half of the fertilized ova or zygotes will again possess six pairs of ordinary chromosomes plus one X, making a total of thirteen. The other half of the zygotes, however, will show the six ordinary pairs and also an X pair; i.e., a total of fourteen. In such cases, it has now been conclusively shown that all the zygotes containing only a single X chromosome will develop into males, while all those with a pair of such chromosomes will become females. In this manner the cycle is continually repeated from one generation to another. From these facts it appears that in such in-stances the factor which determines sex is the presence or absence of certain chromosomes, or at least a certain number of a particular kind

of chromosomes. Because of these facts the X chromosomes are also known as the *sex chromosomes*. Furthermore, the sex which possesses the odd number (usually the male) is said to be *heterozygous*, and the opposite sex *homozygous*, with respect to chromosome number.

It must now be added that numerous variations of the above system occur, which it will be impossible to describe in detail. We may, however, cite a few typical cases. Thus in the insect *Lygaeus*, the female,
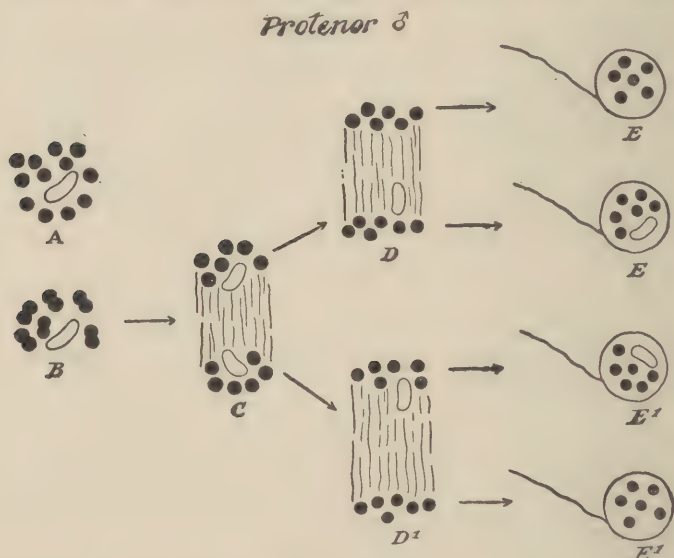
Protenor ♂

Fig. 31. — A diagram of the behavior of the chromosomes during the maturation divisions in the male of *Protenor belfragei*. From Morgan (" Heredity and Sex." Published and copyrighted by the Columbia University Press). The sex chromosome throughout is represented in outline, the others in solid black. A. The chromosomes in the somatic cell of a male. B. The chromosomes united in synapsis prior to the first maturation division of a germ cell. The single sex chromosome is without a mate. C. The first maturation division, which for the sex chromosome is certainly equational. D. The second maturation division, " reductional " for the sex chromosome, i.e., the latter goes to one pole or the other. It is impossible to say certainly in this case which division is really reductional for the ordinary chromosomes (*autosomes*). E, E′. The distribution of the chromosomes in the four spermatids resulting from the two maturation divisions.

as in our first example, has two X chromosomes and the male only one. In this animal, however, the X chromosome in the male does possess a mate, distinguishable from the X in this instance by its smaller size (Figs. 29 and 36). In such cases the mate of the X chromosome in the male is referred to as the Y *chromosome*, and the behavior of the pair

during the maturation divisions is as indicated in Fig. 33. Another instance of this kind is found in the fruit fly, *Drosophila melanogaster*, in which the Y is distinguishable by a hook on one end (Fig. 34A). Many other examples of a similar sort might be given, including Man; in this instance the somatic cells of both sexes appear to possess 48 chromosomes, including an XX pair in the female and an XY pair in the male, in which the Y is again indicated by its smaller size (Painter). A variation of this relationship is seen in *Ascaris incurva*,

Protenor ♀
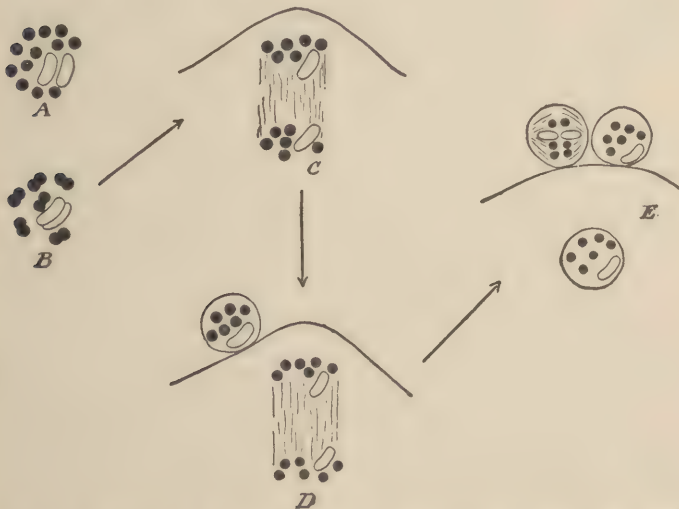
Fig. 32. — A diagram of the behavior of the chromosomes during the maturation divisions in the female of *Protenor belfragei*. From Morgan (" Heredity and Sex." Published and copyrighted by the Columbia University Press). The sex chromosomes throughout are represented in outline, the others in solid black. A. The chromosomes in a somatic cell of the female. B. The chromosomes united in synapsis prior to the first maturation division of a germ cell. Note that in this case the sex chromosome has a mate. C. The first maturation division, probably equational, at least for the sex chromosomes. D. The second maturation division, which, if the first division was equational, is presumably reductional. E. The distribution of the chromosomes in the two polar bodies and the egg. The first polar body is represented as just undergoing the second division.

a parasitic Nematode. Here there are eight X chromosomes and only one Y, the female somatic count thus exceeding the male by seven (Fig. 35).

Another very common situation is that found in certain species of *Drosophila* (not *melanogaster*) and other animals. In these cases the

X and Y are very much alike so far as appearance goes, and in such instances it may become difficult to recognize the sex chromosomes as such. This will obviously be the case if in such a situation these chromosomes also chance to lack any characteristic behavior. Even in such instances, however, there is frequently genetic evidence which
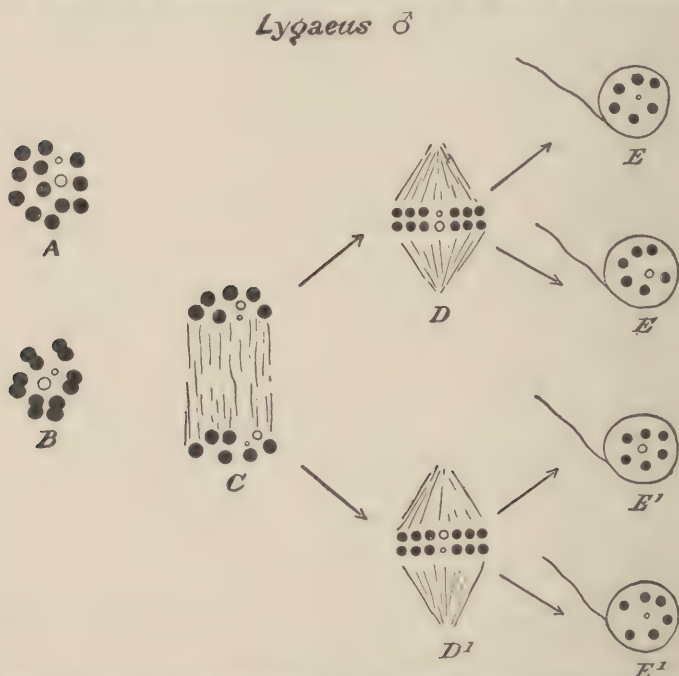
Lygaeus ♂

Fig. 33. — A diagram of the behavior of the chromosomes during the maturation divisions in the male of *Lygaeus bicrusis*. From Morgan ("Heredity and Sex." Published and copyrighted by the Columbia University Press). A. The chromosomes in the somatic cell of a male. Note the large X and the small Y sex chromosomes. B. The chromosomes united in synapsis prior to the first maturation division of a germ cell. The X and Y do not usually unite at this time so that it is not indicated in the diagram (see figure 36). C. The first maturation division in this case, so far as the sex chromosomes go, is evidently equational. D. The second maturation division, which for the sex chromosomes is evidently reductional. E, E'. The distribution of the chromosomes in the four spermatids resulting from the two maturation divisions, two receiving an X chromosome and two a Y.

gives good reason to believe that in the female and male respectively, chromosomes are present which represent as regards their qualities and significance an XX and an XY pair.

Finally the fact may be mentioned that in a few instances the female is believed to contain the odd somatic number; i.e., to be

heterozygous in this respect, whereas the male contains the even number and is therefore homozygous. Hence, it follows that there are two kinds of eggs and one kind of sperm. This is thought to be the case with the moth *Abraxis*, and this arrangement is therefore referred to as the *Abraxis* type.

In conclusion of this topic it may be stated that a very large number of cases fundamentally similar to those just described are now known
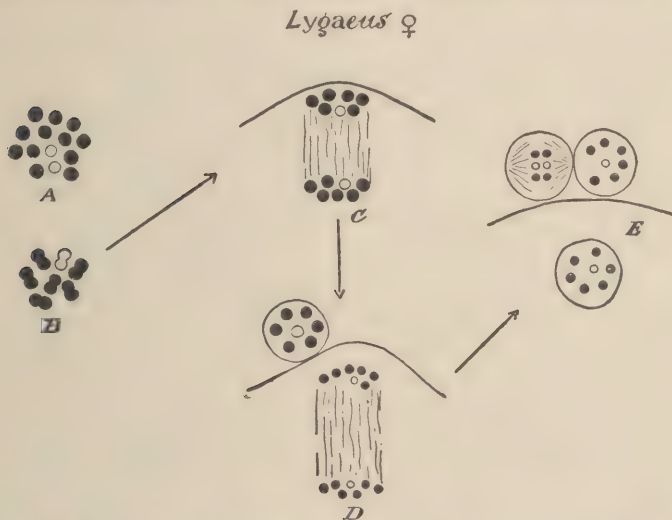
Lygaeus ♀

Fig. 34. — A diagram of the behavior of the sex chromosomes in the female of *Lygaeus bicrusis*. From Morgan (" Heredity and Sex." Published and copyrighted by the Columbia University Press). A. The chromosomes in the somatic cell of a female. Note the two X chromosomes. B. The chromosomes united in synapsis prior to the first maturation division of a germ cell. C. The first maturation division, probably equational. D. The second maturation division, probably reductional. E. The distribution of the chromosomes in the two polar bodies and the egg. The first polar body is just undergoing the second division.

to exist among animals, as well as a few among plants. Upon this basis, therefore, it has been maintained by many that the determination of sex is entirely dependent upon internal factors; i.e., upon the random mating of germ cells containing or lacking certain chromosomes; hence the outcome can in no way be influenced by any environmental changes. Thus Professor T. H. Morgan may be quoted upon this point as follows: "At best the environment may slightly disturb the regular working out of the two possible combinations that

give male or female. Such disturbances may effect the sex ratio, but have nothing to do with sex-determination." As a result of further investigation, however, it now appears that at least in some instances external as well as internal factors may be active. Hence in certain cases, which thus far seem to be relatively exceptional, a partial environmental determination of sex may after all be possible.

The Reasons for Belief in a Reductional Maturation Division. — It is quite possible, indeed certain in many cases, that the mechanism of maturation does not work in just the way that has been described. In discussing the subject, however, the belief has been expressed that

Fig. 34A. — From the " Mechanism of Mendelian Heredity," after Bridges. The female and male groups of chromosomes in *Drosophila melanogaster*, showing the four pairs of autosome chromosomes plus the XX pair in the female and the XY pair in the male. In this animal the members of each pair are usually found together as indicated.

one of the divisions involved is always reductional; i.e., that it results in the separation of the members of chromosomal pairs which have been temporarily fused. Having now finished our account of the peculiar sex chromosomes, it is possible to give some reasons for this belief. They are as follows:

I. There is the behavior of the sex chromosome in those instances where it lacks a mate. In such cases as has been seen it only divides once, while all the other chromosomes which have mates divide twice. This fact is quite striking. It is easily and obviously explained, however, by assuming that one of the divisions of the paired chromosomes is for the purpose of separating one member of a pair from

another. In that case, since the sex chromosome is single, it would of course divide but once.

II. There is, furthermore, the behavior of the sex chromosomes in females and also in those males where both an X and a Y are present. Here as noted the members of the sex pair resemble their fellows in that they also meet in a kind of synaptic union. In many

Fig. 35. — Polar views of chromosome groups in the maturation divisions of the spermatocytes in *Ascaris incurva*. After H. B. Goodrich. A. A metaphase plate in the first spermatocyte division, which as regards the sex chromosomes in this animal is certainly reductional. Note the group of eight X chromosomes in the center of the plate. The Y chromosome is in the circumference and almost in contact with one of the long X chromosomes with which it was mated. B. An anaphase plate of the first spermatocyte division, viewed from the pole toward which the Y chromosome is being drawn. The other halves of the autosomes and the group of X chromosomes have gone toward the other pole and are therefore not visible. C. A metaphase plate of the second spermatocyte division in the case of a cell which has received from the first division 13 autosomes and the Y chromosome. The latter is not identified in this figure. D. A metaphase plate of the second spermatocyte division in the case of a cell which has received from the first division 13 autosomes and the 8 X chromosomes. The latter are again visible as a group in the center of the plate, save for one of the very small X chromosomes which is in the margin.

instances, however, as already stated, the sex chromosomes exist at this time not as mere threads, but as well-defined rods or spheres. Also, the degree of synaptic union in the case of these bodies is often very slight (Fig. 36). Under such conditions, it is much easier to be sure of the exact nature of the division which follows. As a result,
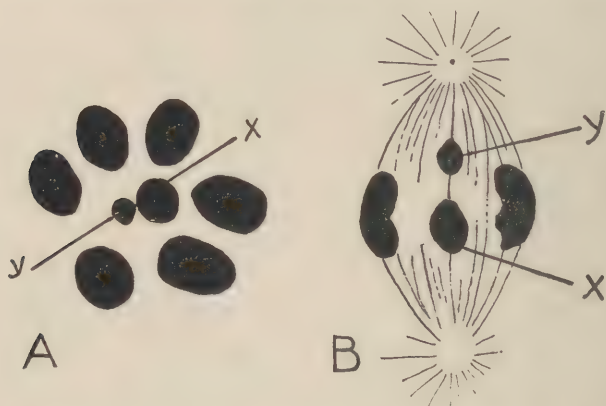
Fig. 36. — Division figures from the maturation of the germ cells in the male of *Lygæus bicrusis*. After E. B. Wilson. A. A polar view of the first maturation division. In this insect the synapsis of the X and Y chromosomes not only does not occur while they are in a thread-like condition, but is postponed until almost the end of the first maturation division. Even then it is evidently very slight, as indicated by the figure. B. A side view of the second maturation division in the same animal. The chromosomes in this case do not lose their identity during interkinesis (i.e., the interval between the two divisions), and it therefore is possible to determine that the X and Y which united in synapsis at the end of the first division, as shown in A, are now being separated from one another. Thus for these chromosomes in this instance the second division is clearly reductional.

the opinion of competent observers who have studied this division is significant, and this opinion is that, to all appearances, the division in question is reductional. It would then be expected in such instances that the other maturation division of these chromosomes, whether first or second, would be equational, and apparently it is so. There are various stages, however, which intervene between such clear cases and those in which sex chromosomes, like ordinary chromosomes, mate only as threads. Yet there is little reason to suppose that in the case of the sex chromosomes there is any fundamental difference between these two forms of the process. If then this argument applies to the sex chromosomes, it would seem to apply equally well to the others whose usual synaptic condition is threadlike.

III. Finally, there is the case of maturation in hybrids, where the two parents have contributed unequal numbers of chromosomes. Here there are again one or more chromosomes without mates, just as in many normal cases of the sex chromosome. As in those cases, these extra chromosomes divide only at one of the maturation divisions, and the same explanation and argument applies as was stated under (1). An excellent example of this is the case of the cross between *Drosera rotundifolia* and *D. longifolia,* made by Rosenberg. The mature gamete of the former plant has 10 chromosomes and that of the latter 20. Thus the somatic cells of the hybrid contain 30 chromosomes. Upon maturation of the hybrid germ cells, however, there do not appear, 15 bivalent chromosomes; instead there are only 10 bivalents, plus 10 univalent or single chromosomes. The reason obviously is that there are only 10 chromosomes of *D. rotundifolia* to be mated, thus leaving 10 of the 20 chromosomes of *D. longifolia* unmatched. Under such circumstances as would be expected, the 10 single chromosomes divide at only one of the two reduction divisions. Geertz also obtained similar results in a cross between *Oenothera Lamarkiana* with 7 chromosomes in the gamete and *Oe. gigas* with 14, while more recently (1919), Kihara has found the same type of phenomena in a wheat hybrid.

THE SIGNIFICANCE OF MATURATION

This problem may be attacked by considering first the apparent results of this process. It will then be possible to state briefly in what way these results are significant, and finally to offer some further evidence for belief in the statement.

The Results of Maturation. — The results of maturation may be summed up in four statements, as follows:

I. There is in connection with the cell divisions of this period an obvious *reduction in the amount of chromatin.* It should be noted, however, that a mere reduction in the quantity of this substance often occurs in a much less elaborate manner. Thus, as has been indicated above, the oöcyte during its growth frequently eliminates chromatin by casting it out into the cytoplasm.

II. There is during synapsis a *reduction in the number of chromosomes,* thereby avoiding a doubling of this number during fertilization, even though no division of the cell were to follow.
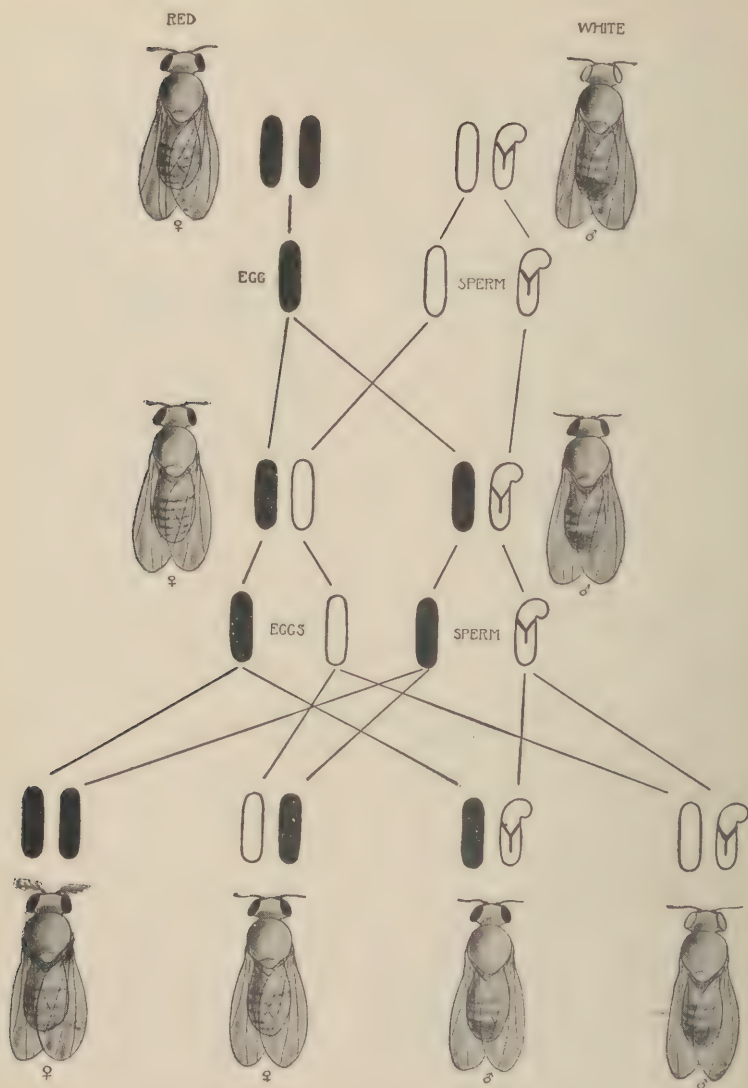
Fig. 37. — From Morgan (Mechanism of Mendelian Heredity). Red-eyed female by white-eyed male (*D. melanogaster*). The genes for these characters are carried by the X chromosomes, a gene for red by each X chromosome represented in solid black, and a gene for white by each X chromosome represented in outline.

III. There is also during synapsis the apparently *close union of the members of chromosomal pairs*, each member of every pair having been derived from one of the parents of the animal in question. Thus there is afforded the opportunity for the exchange of material between the members of every such pair.

IV. Following synapsis there is, according to the evidence given above, a *true reduction division*. Granting that such is the case, the evident outcome will then be the distribution of one complete haploid set of chromosomes to each germ cell, except, of course, in the case of the odd sex chromosomes.

These then are the most prominent, and presumably most important results of maturation. Of these results, moreover, it will be noted that the last three are entirely peculiar to this process, while the first is peculiar only as regards the manner of its occurrence.

The Significance of these Results. — The question now remains as to whether these results are of especial importance, and if so in what way. This question may be briefly answered by the following statement. In a preceding section much emphasis was placed on the point that the facts of heredity could best be explained on the assumption that chromosomes are the bearers of genes or determiners of hereditary traits. *It may now be added that this assumption, which so well explains hereditary phenomena, demands further just such a process as appears actually to be furnished by maturation.*

Furthermore, since the student is by this time familiar with the details of the last named process, as well as with the character and behavior of the sex chromosomes, it is now possible to demonstrate the truth of the foregoing statement by an extremely impressive illustration.

Sex Linked Inheritance. — Sex linked inheritance is so named because the peculiar method in which the characters are inherited is entirely accounted for by merely assuming their genes or determiners to be carried exclusively by the sex chromosomes. That is, it has been discovered that the distribution of these chromosomes during maturation and fertilization, according to the above interpretation of these phenomena, perfectly corresponds with, and therefore explains, the accompanying distribution of the characters. The consideration of a particular case of this type of inheritance will now serve to make these points more clear.

Sex Linked Inheritance in Drosophila Melanogaster. — In the

case of the fruit fly, *Drosophila,* the eyes are normally red.   Professor
T. H. Morgan discovered in his laboratory, however, a male fly
which possessed white eyes.   From this fly by proper breeding
he then obtained a white-eyed race, including both males and fe-
males.   The breeding, moreover, brought out the following facts re-
garding the inheritance of the character in question.

If a red-eyed female was mated to a white-eyed male (Exp. I), the
first generation of flies all had red eyes.   If the brothers and sisters of
this brood were then bred with each other it was found that in the
next generation all the females and approximately half the males had
red eyes.   The remaining half of the males, on the other hand, had
white eyes.   If, however, the cross of the parent flies was reversed as to
sex; i.e., if white-eyed females were mated to red-eyed males (Exp.
II), the result appeared thus: in the first generation all the females
were red-eyed and all the males white-eyed; if these were then inbred
as in the first case, the second generation produced approximately half
of each type of flies in each sex.

Let us now turn to the supposed behavior of the sex chromosomes
in these cases in order to see what light their distribution throws
upon this curious phenomenon.   This can be most easily demonstrated
by reference to two diagrams (Fig. 37 for Exp. I; Fig 38 for Exp. II).
Before the solution which these diagrams afford will become evident,
however, a little explanation in relation to them is required.

In each diagram the parent flies and the two generations to which
they give rise in each of the experiments are represented respec-
tively in three rows, one beneath the other.   In every case the number
of insects pictured is only enough to indicate all the types of flies, male
and female, which occur in that particular generation.   Thus there
are two flies in each of the first two rows and four in the last.   The
sex of each fly is shown by the usual conventional signs, ♂ for the
male and ♀ for the female, placed just beneath the animal, while
the eye color is indicated by black for red eyes and light gray for
white eyes.   Furthermore, in connection with the pictures of the
flies, the sex chromosomes and their behavior are designated as fol-
lows: Beside each fly in the first two rows and above each in the
last, there are represented in conventional fashion the two sex chromo-
somes which the somatic cells of that fly are assumed to contain.   The
X chromosomes are pictured as straight rods, the black ones carrying
the gene for red eyes and the plain white ones the gene for white
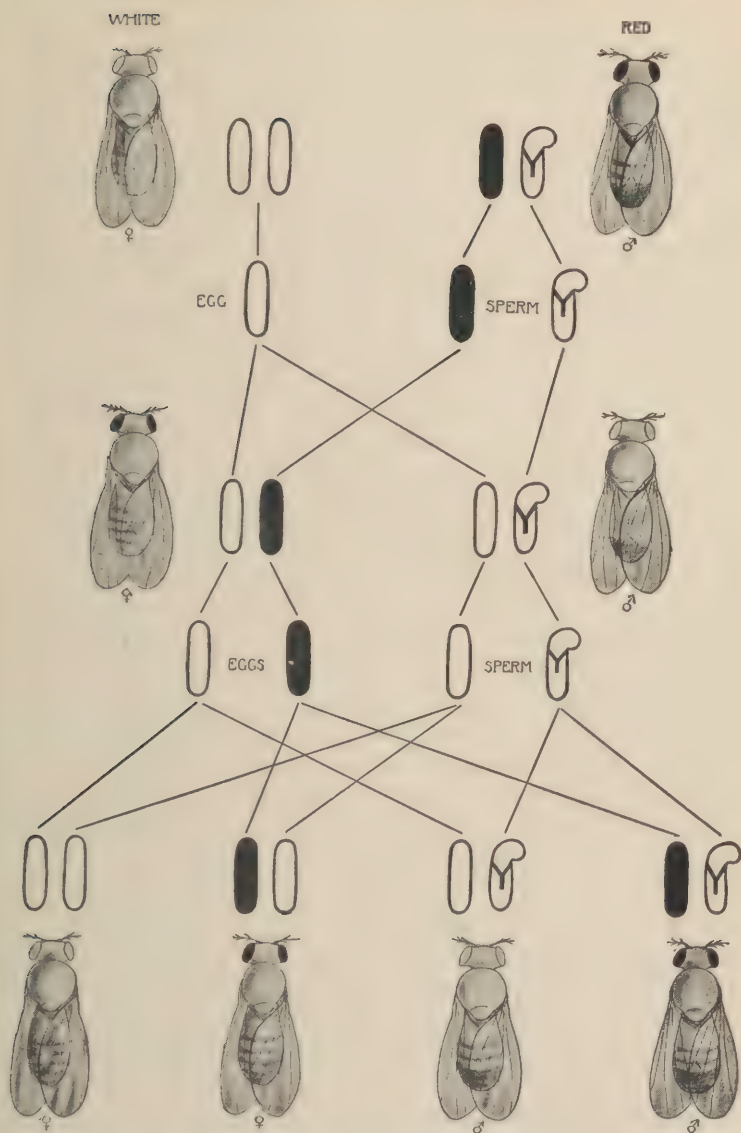
Fig. 38. — From Morgan (Mechanism of Mendelian Heredity). White-eyed female by red-eyed male (*D. melanogaster*). This is the reciprocal of the cross shown in Fig. 37.

eyes. The Y chromosomes on the other hand are represented as white rods bent at one end and marked with the letter Y. So far as is known from genetic evidence these chromosomes in *Drosophila* appear to carry no genes for anything, with one possible exception to be mentioned in connection with another experiment. On this basis the diagram shows, as would be expected, that every somatic cell of each female fly contains two X chromosomes, and every somatic cell of each male an X and a Y. Likewise, besides the above sex chromosomes which the somatic cells of the animals contain, there appear immediately beneath the first and second rows of flies the kinds of sex chromosomes which will occur in the eggs or sperm of these flies after maturation. There will of course be only one such chromosome in each spermatozoön or egg, but, as usual, in the case of the spermatozoa this chromosome may be either an X or a Y. Finally in these experiments, as indicated, the X chromosome may carry either the gene for red or for white eyes.

One further word is now needed concerning the genes themselves and their apparent effect upon the flies. It is quite evident that if the chromosomes carry genes which determine characters, when animals with opposite characters are crossed the cells of the offspring must contain one gene of each kind; i.e., they are said to be heterozygous [5] as regards these factors. However, as is evident in the case under consideration, the genes are apparently not equally potent in their influence on the character which they determine. If they were so, a blend might result, and in some cases of heredity this happens. In this instance, however, the influence of one gene (i.e., the gene for red eyes) dominates the other, and the character in question is then said to be *dominant* over its opposite, which in turn is said to be *recessive* (e.g., note the female in the second row, Fig. 38). It should be sharply emphasized, however, that whatever the influence of the genes on the characters of an individual may be, it is believed that the genes themselves *never influence each other*. Thus no matter how close their contact during maturation, at the end of that process each gene itself remains unchanged.

[5] The terms *homozygous* and *heterozygous* are here used as indicating that the two members of a pair of genes which are concerned with the determination of a given character are respectively similar or dissimilar to one another. They do not, in this case, refer to the odd or even number of the chromosomes as on page 58.

With these facts in mind it is now possible to interpret the above experiments in terms of the diagrams.

In the first experiment (Fig. 37) it will be recalled, the female had red eyes, and the male white eyes. Furthermore, the female is assumed to be pure, or homozygous, as regards the gene for red eye color, and hence its sex chromosomes are indicated by two dark rods. The male on the other hand, being white eyed, its single X chromosome is pictured as white. Following now the distribution of the sex chromosomes, it is apparent that in the first generation the females will have one X chromosome with a gene for red and one with a gene for white eyes (i.e., they will be heterozygous as regards this gene), while the males will contain a single gene for red eyes. This is because a male always necessarily gets its single X chromosome from its mother, while a female gets one of her two sex chromosomes from each parent. As regards the results of this distribution of genes upon the characters, it is evident that since the gene for red eyes is dominant the heterozygous females will all have eyes of that color. So likewise will all the males whose single X chromosome in this instance also carries the gene for red eyes. With this demonstration to start with, the student should now be able to follow the distribution of the chromosomes the remainder of the way through both experiments. When this is done, moreover, it will appear, as indicated, that the actual facts of inheritance of this character as first stated are perfectly accounted for by the order of this distribution.

This example illustrates the simplest instance of the type of inheritance under discussion. It is important to note, however, that certain variations of this and other types not infrequently occur, whose explanation in connection with the cytological facts of maturation, is not without significance. Briefly stated, this explanation involves the assumption that the genes contained in one member of a pair of chromosomes sometimes change places with those which occupy a corresponding place in the other member of that pair. The importance of this point will be realized when it is remembered that an opportunity for just such an exchange of material appears to be afforded during the intimate union of the chromosomes in synapsis. In other words, the facts of genetics seem to continue to bear out those of cytology so far as the latter are known. Fig. 38A indicates diagrammatically the manner in which the above exchange is thought to take place, and suggests the reason for the term *crossing over* which has been applied to it.

Non-Disjunction in Drosophila. — It finally remains to mention very briefly another much more unusual variation of the normal sex linked ratio in *Drosophila*. This varia-

tion, while at first appearing to upset the above chromosomal explanation, ended by confirming it in a most striking manner. For reasons which will presently appear, it was called *non-disjunction,* and was discovered by one of Professor Morgan's students, Dr. C. B. Bridges. It was found that in some instances when an apparently normal white-eyed female was mated to a red-eyed male, a few of the daughters (about 2.5%) would be white-eyed instead of all red as expected. Also a corresponding per cent of the males would be red-eyed instead of all white-eyed. To explain this variation, Dr. Bridges conceived the idea that at the reduction division in the germ cells of the parent white-eyed female, the two sex chromosomes failed to separate; i.e., to " disjoin." If this in fact occurred, it would give rise to two possibilities. The two X chromosomes might both stay in the egg, or they might both pass out in the polar body. In those eggs in which the former event occurred there

Fig. 38A. — Diagram to illustrate crossing over. From Morgan (Mechanism of Mendelian Heredity). The white and the black rods (*a*) twist and cross at two points (*b*). Where they cross they are represented as uniting (shown in *c*). That an interchange of pieces has taken place in the region between genes *W* and *Br* is demonstrated from the standpoint of inheritance by breeding experiments. The results of these are most readily explainable on the assumption that the gene *M* has gone over to the other chromosome.

would remain two X chromosomes, and if such an egg was then fertilized by a sperm bearing a Y chromosome the result would be a white-eyed daughter. This follows since the Y carries no genes, or at least none which have any bearing on this character. In this manner the occurrence of the first of the above possibilities would account for the exceptional females. On the other hand, in those eggs in which both the X chromosomes passed out, it is evident that fertilization by an X bearing sperm must result in a fly whose cells have one X chromosome with a gene for red eyes. Since there is only one X, moreover, this animal would naturally be a male, and thus the presence of the unexpected red-eyed flies of the latter sex is also

accounted for. The distribution of the chromosomes in the matings just described is illustrated in Fig. 39.

Although the above assumptions offered a solution to the variations noted, it was still necessary, if possible, to verify them. Such verification was at first attempted by further breeding, and the results obtained were just those which were to be expected if the above
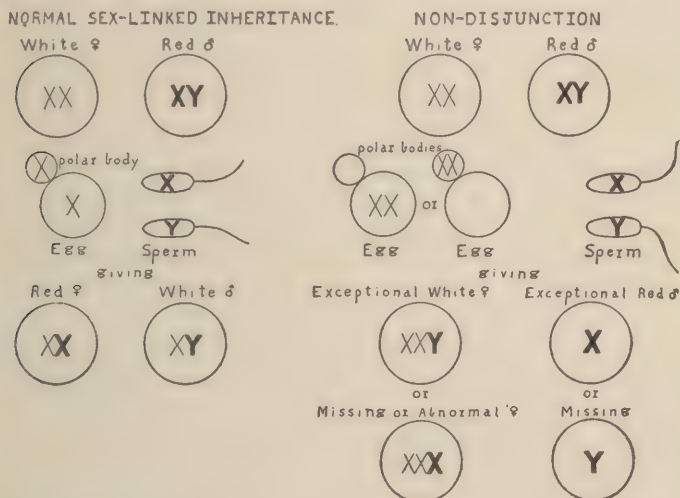
Fig. 39. — Diagram illustrating sex-linked inheritance in which the two X chromosomes in the eggs of some individuals (those on the right) fail to separate at maturation; i.e., non-disjunction occurs. In this figure only the sex chromosomes are indicated throughout. Each X in heavy type represents a sex chromosome carrying a gene for red eyes, and each X in light type represents a sex chromosome with a gene for white eyes. Since the gene for red eyes is dominant, an individual with genes for both white and red eyes possesses red eyes. The Y chromosome, as usual, carries nothing known to produce any somatic effect.

hypothesis were correct. Far more striking, however, has been the verification revealed by a cytological examination of the exceptional white-eyed females and the red-eyed males. Thus the cells of the former, it will be recalled, were assumed to possess the sex chromosome formula XXY instead of the normal XX, whereas those of the latter were supposed to have the formula XO instead of the normal XY. It may now be stated that a study of the cells of these forms has shown that *just these chromosomal relationships actually exist.*

These results are probably the most fundamental ones connected with this experiment. There are, however, certain other suggestive features which are worth noting. Thus, although the flies having the

XO complex appeared perfectly normal as regards their somatic characters, they were nevertheless sterile, a condition which cytological examination showed to be due to defective germ cells. Hence, as previously intimated, the Y chromosome seems to contain at least one determiner; i.e., the one which prevents the type of sterility just indicated. Even so, the importance of this chromosome, so far as the development of the soma is concerned, is negligible. This becomes evident upon consideration of the following facts: (1) Although the XO type of male is well known, the YO type, which on the basis of chance distribution should be equally frequent, is actually never found. This of course is to be expected on the assumption that the Y chromosome lacks those determiners which are essential for development. (2) The white-eyed XXY females noted above are quite normal in appearance despite the presence of the Y, indicating that the latter is without somatic influence. (3) The XXX type of red-eyed female, on the other hand is rarely viable, and when it is so it always possesses certain specific peculiarities. Furthermore, by proper crossing it is possible to obtain an XYY individual which, besides being a male, is as regards its soma, entirely normal; i.e., an extra Y is again apparently without effect, while an extra X produces well-defined disturbances.

General Conclusions. — Upon the basis of these facts of heredity and cytology as illustrated by *Drosophila*, therefore, it would seem to be virtually demonstrated that the sex chromosomes are actually the bearers of hereditary genes or determiners. Such at least must be admitted for the particular case in question, and it may be further added that numerous other instances of ordinary sex linked inheritance are well known. Thus in man, for example, color blindness, hemophilia, and night blindness all appear to be inherited according to this plan, presumably through the agency of a sex chromosome. As has already been suggested, however, these instances of sex linked inheritance are only a few of the facts of heredity which can be explained on the so-called chromosomal hypothesis. Numerous others not of the sex linked variety (e.g., the occurrence of the well known 3 to 1 Mendelian ratio and its variations) can be equally well accounted for by extending the application of the hypothesis to the remainder of the chromosomes. The scientific basis for the interpretation of the specific character of the chromosomes, and the entire phenomenon of maturation as above presented, would seem to be extremely well grounded. (References to the literature will be found at the end of Chapter II).

FERTILIZATION AND EARLY STAGES IN DEVELOPMENT

FERTILIZATION

Before proceeding to an account of development in any particular animal, it may be well first to discuss in a general way certain of the earlier processes which are always involved, and to note the chief methods of their occurrence.

Fertilization in all higher forms consists of the union of an egg and a spermatozoön. This union may occur within some cavity of the female into which the sperm have been introduced, or it may occur outside. The latter is the more common method among animals which live in the water. In either case, thousands of the relatively minute sperm are required to insure the fertilization of each single egg by one spermatozoön. We shall now turn to a generalized account of the process.

PENETRATION

The Action of the Sperm. — According to some writers the egg secretes a substance which tends to attract the spermatozoa to its surface. This, however, is very doubtful. At all events, as the male reproductive cell moves head foremost, propelled by the swimming movements of its tail, the acrosome first comes in contact with the egg, and may in some cases aid in penetrating its membrane. This penetration may take place at any point of the surface in case the membranes are thin, or the sperm may be compelled to enter through a special orifice, the *micropyle*. Usually only one sperm enters (*monospermy*); in case more do so, the development is generally abnormal. In some instances, however, especially in eggs with a large yolk, it is normal for several sperm to enter, and the phenomenon is known as *polyspermy*. Even in those cases where the latter condition occurs,

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however, it will appear later that only one of the spermatozoa takes active part in the further events of actual fertilization. The remainder eventually degenerate and disappear, though previous to this they may divide several times, and perhaps aid in breaking up the yolk to make it more easily assimilable. In such cases they are referred to as *merocytes*. The method by which the extra sperm are excluded in the event of monospermy will be discussed presently.

As soon as the head of the sperm has punctured the surface of the egg, the swimming movements of its tail cease. In some cases the latter is regularly drawn into the egg along with the head and middle piece, while in others it is left outside. In either event it soon degenerates and takes no more part in the fertilization process.

The Reaction of the Egg.

The Perivitelline Space and the Fertilization Membrane. — Probably the first and most characteristic reaction of almost all eggs to puncture by a sperm is the formation of a space between the egg surface and its innermost covering (i.e., in most instances the vitelline membrane). It is called the *perivitelline space* and seems in some cases to be due to the pushing away of the membrane by a secretion from the egg. In other instances it may be due to shrinkage of the egg or to absorption of water by some substance between the membrane and the egg surface. In any event such a separation of the egg from its covering of course makes the latter more conspicuous, and even in such eggs as have seemed previously to lack a membrane, one now becomes visible. Because of this increased visibility following fertilization, the membrane about the perivitelline space, whether it be the original vitelline membrane, one apparently newly formed, or a fusion of both of these, is frequently called henceforth the *fertilization membrane* (Fig. 54, *D*). The significance of the phenomenon just noted is not well understood. It was thought at one time to aid in preventing polyspermy. Since eggs from which the membranes have all been entirely removed continue to be impervious to further fertilization, however, it is evident that this condition is not the result of the existence or the location of any membrane. It has also been maintained that the obvious alteration in position of the membrane is accompanied by increase in its permeability to gases and other substances. That there is considerable basis for this belief is indicated by the fact that in some instances there is a decided increase in oxidative processes and other phenomena requiring such a change.

The Changes in the Egg Cytoplasm. — Aside from these phenomena connected with the inner egg membrane, fertilization also initiates certain other changes in the egg proper. Almost simultaneous with the appearance of the perivitelline space there is frequently evident an outpushing of the cytoplasm at the point where a spermatozoan has penetrated the fertilization membrane. This protuberance is then entered by the sperm, and because of this fact it is often termed the *entrance cone* (Fig. 40, *B*). Following these events both the cone and the parts of the sperm which it contains are apparently drawn down
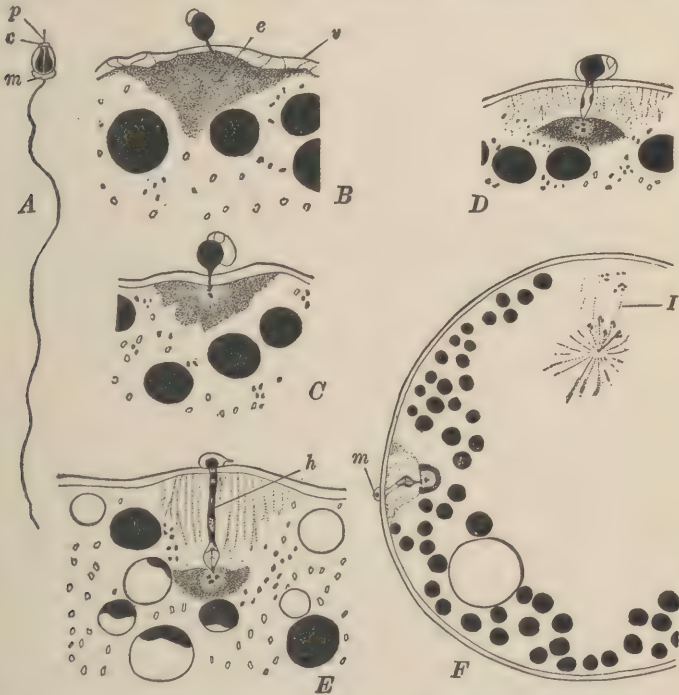
Fig. 40. — Entrance of the spermatozoön in the fertilization of the Annulate, *Nereis limbata*. From Kellicott (General Embryology). After Lillie. A. Spermatozoön. B. Perforatorium has penetrated egg membrane; entrance cone well developed. Fifteen minutes after insemination. C. Thirty-seven minutes after insemination. D. Entrance cone sinking in and drawing the head of the spermatozoön after it. Forty-eight and one-half minutes after insemination. E. Head drawn in still further. Forty-eight and one-half minutes after insemination. F. Entrance completed. First maturation division in anaphase. Fifty-four minutes after insemination. The middle piece, as well as the tail, remains outside.

c. Head cap. *e.* Entrance cone. *h.* Head of spermatozoön (nucleus). *m.* Middle piece. *p.* Perforatorium. *v.* vitelline membrane. *I.* First polar division figure.

into the deeper egg substance (Fig. 40, *C*, *D*, *E*). Besides this some-
what localized activity on the part of the cytoplasm, however, there
are also evidences of other effects which seem to be more widespread.
Thus, since polyspermy is not prevented by the fertilization mem-
brane, it is held that such prevention may be due to a general altera-
tion in the egg cytoplasm. More specifically, it is thought that the
same stimulus which causes the formation of the perivitelline space
also causes the inactivation of some chemical agent (*fertilizin*) pos-
sessed by the egg, without which it can not effectively react to the
sperm (Lillie). Whether or not this be true, evidence is not wanting
that in some cases at least all of the egg cytoplasm is profoundly
disturbed by the sperm entrance. It seems likely indeed that this is
more or less true of all eggs, but the disturbance is particularly
obvious in certain instances because in these instances different re-
gions of the egg cytoplasm are differently colored and thus distinguish-
able.   In such eggs it has therefore been possible to observe that
following fertilization a sudden and marked rearrangement of these
parts of the cytoplasm takes place.   Such, for example, is the case
with the egg of the Tunicate, *Cynthia* (*Styela*) *partita* (Fig. 41).

THE LATER STAGES

The later steps in the fertilization process which are now to be
described are all more or less directly connected with the fusion of
the nuclei of the sperm and egg.

The Egg Nucleus. — The maturation divisions of the egg are some-
times entirely completed previous to fertilization. More usually, how-
ever, as in the case of most Vertebrates, only one of these divisions
occurs before the sperm entrance, and in some instances (e.g., *Nereis*)
both are delayed until after this event (Fig. 43, *B*, *C*).   In these
cases where maturation has not begun, or is unfinished prior to the
penetration of the sperm, the latter event seems to act as a stimulus
which causes the maturation to proceed. As soon as it is completed
the egg nucleus is definitely formed, and the centrosome which took
part in the second division disappears.

The Sperm Nucleus and the Centrosome. — While this comple-
tion of the maturation divisions is taking place the head and the middle
piece [1] of the sperm advance into the egg. Also, as this occurs these

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[1] In some instances; e.g., Nereis, the middle piece, as well as the tail, remains
outside.
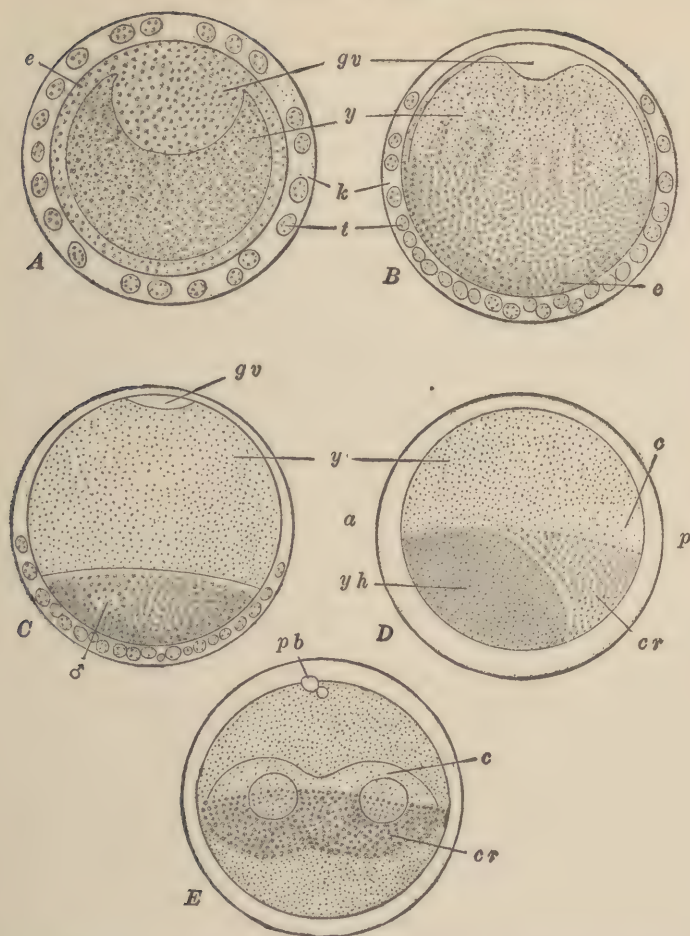
Fig. 41. — Total views of the egg of the Tunicate *Cynthia partita*, showing the changes in the arrangement of the materials of the egg subsequent to fertilization. From Kellicott (General Embryology). After Conklin. x. 200. A. Unfertilized egg, before the fading out of the germinal vesicle. Centrally is the mass of *gray* yolk; peripherally is the protoplasmic layer with *yellow* pigment, and surrounding the egg, the test cells and chorion. B. About five minutes after fertilization, showing the streaming of the superficial layer of protoplasm toward the lower pole where the spermatozoön enters, and the consequent exposure of the gray yolk of the upper hemisphere. The test cells are also carried toward the lower pole. C. Side view of eggs showing the yellow protoplasm at the lower pole; at the upper pole a small clear region where the polar bodies are forming. The location of the sperm pronucleus (nucleus) is also indicated. D. Side view of egg shortly before the first cleavage, showing the posterior collection of the pigmented protoplasm (yellow crescent) and the clearer area above it. E. Posterior view of egg during the first cleavage, showing its relation to the symmetry of the egg.

a. Anterior. *c.* Clear protoplasm. *cr.* Yellow crescent. *e.* Exoplasm or cortical layer, with yellow pigment. *g.v.* Germinal vesicle. *k.* Chorion. *p.* Posterior. *p.b.* Polar bodies. *t.* Test cells. *y.* Yolk (central gray material). *y.h.* Yellow hemisphere. ♂ Sperm nucleus.

parts rotate through an angle of 180° so that the middle piece is in the lead (Fig. 42, *A*, *B*). The advance then continues along a course whose first portion is called the *entrance* or *penetration path*, and which, in the case of the Frog, is marked by granules of pigment. Meanwhile the acrosome which effected the entrance of the sperm has disappeared, while marked changes are also taking place in the nuclear portion of the head and the middle piece. The former is enlarging, and within it the chromatin is forming a typical nuclear reticulum. In the region of the middle piece, on the other hand, a centrosome appears and is presently surrounded by a small aster. It has been claimed that this centrosome is identical in whole or in part with the centrosome which formed, or at least entered, the middle piece during the transformation of the spermatid. This is very doubtful, and in many cases is certainly not true. It does seem, however, that in most instances the new centrosome at least arises under the influence of the middle piece.

The Fusion of the Egg and Sperm Nuclei. — Previous to or during the above processes, the second maturation division of the egg has been concluded, and the egg nucleus has moved from the periphery of the cell into approximately the midst of the active cytoplasm (Fig. 42, *D;* Fig. 43, *D*). Of course in telolecithal eggs with a large yolk, this point will be just below the surface of the animal pole, rather than at the actual center of the egg. The new sperm aster and nucleus, which have meanwhile been advancing along the penetration path, now move directly toward the egg nucleus. This in many instances may involve a slight change in the course of the sperm, and when such is the case the latter portion of its course is termed the *copulation path* as distinguished from the first portion or entrance path (Fig. 42, *C*).

As the nuclei meet each other their membranes disappear. Also there has appeared in each the haploid number of chromosomes [2] (Fig. 42, *C*, *D*). Meanwhile the sperm centrosome and aster divide, if indeed they have not already done so, and form a typical division spindle. Upon this spindle the restored number of chromosomes arrange themselves, and each is then divided in the usual manner preparatory to the first cleavage of the egg (Fig. 43, *E*). It should be

[2] In many cases the chromosomes are not actually visible as such until after the fusion of the pronuclei. In these instances the number appearing in the single fusion nucleus is then diploid as would be expected.
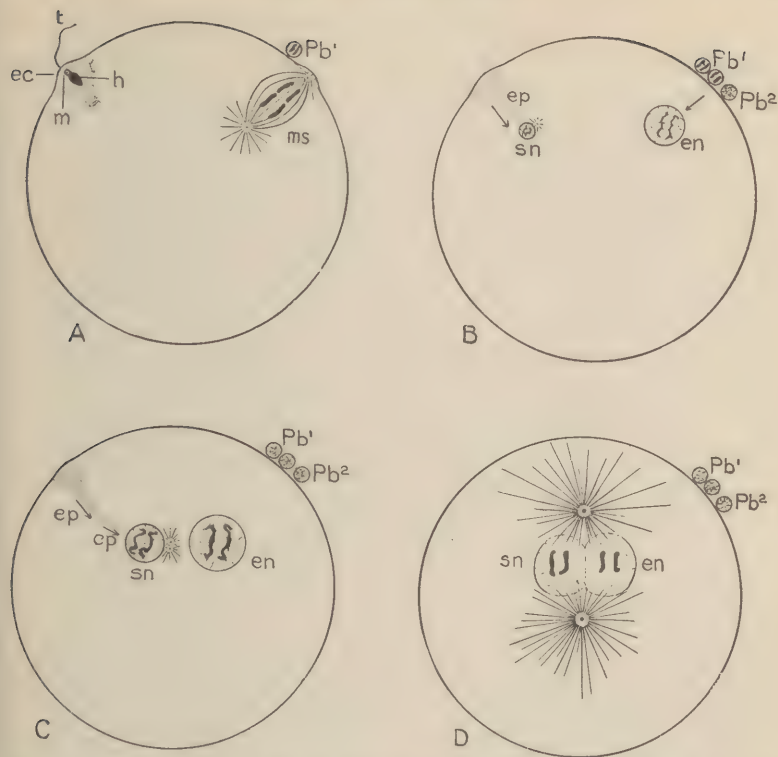
Fig. 42. — A generalized diagram of the penetration of the sperm and the fusion of the egg and sperm nuclei, the haploid number of chromosomes being assumed in this case to be two. The trail of pigment marking the path of the sperm actually occurs only in the case of the Frog's egg. The egg membranes are not represented. Compare the stages with those in Fig. 43 showing corresponding processes in the egg of Nereis.

A. The first polar body has been given off, and the second maturation division is in progress. The sperm head and middle piece have entered the egg, leaving the tail outside. B. The first polar body has divided and the second has been given off, while the completed egg nucleus has started to move toward the center of the ovum. The sperm nucleus consisting of the sperm head has enlarged somewhat, has partially rotated, and is also moving toward the center of the egg. The centrosome has appeared in the region of the middle piece. C. The two nuclei are enlarging and approaching one another. The sperm nucleus, having completed its rotation, has altered the direction of its movement somewhat (not always necessary), to hasten their meeting, and the centrosome is dividing into two parts. D. The nuclei, each containing the haploid number of chromosomes, have started to fuse. The centrosomes, each surrounded by a centrosphere and aster, have taken up their places preparatory to the first division of the egg.

cp. Copulation path. ec. Entrance cone. en. Egg nucleus. ep. Entrance path. h. Head of sperm. m. Middle piece of sperm. ms. Maturation spindle of the second maturation division. pb1. pb2. First and second polar bodies. sn. Sperm nucleus. t. Tail of sperm.
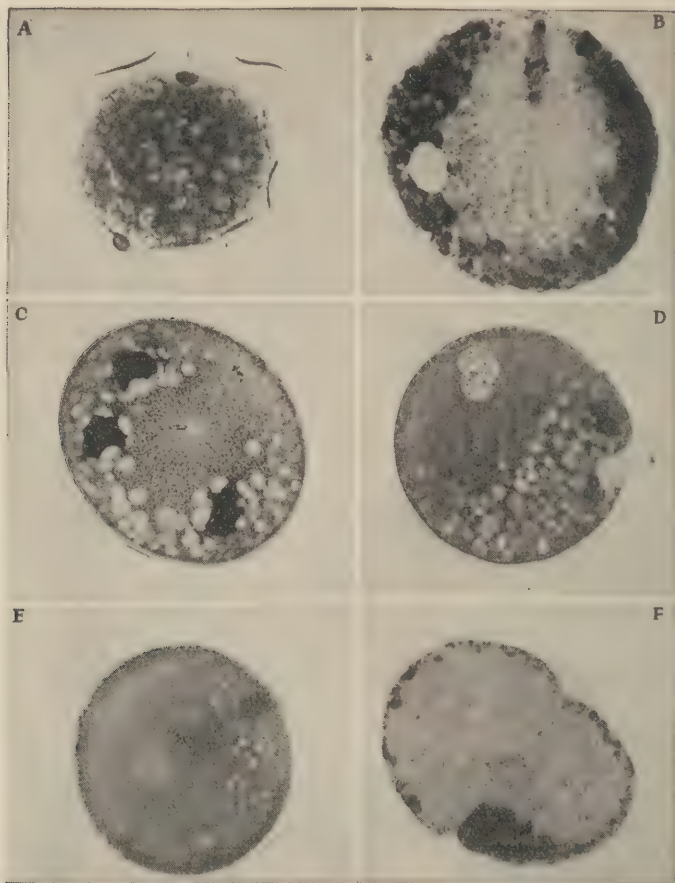
Fig. 43. — Microphotographs of sections of *Nereis* eggs, showing stages in fertilization maturation and cleavage. The photographs were made in the Anatomical Department of Western Reserve University Medical School from specimens presented to that department by Professor O. Van der Stricht, and are reproduced by the courtesy of Professor Van der Stricht and Dr. E. W. Todd.

A. At the top of the figure the spermatozoön is shown just entering the egg. The egg membrane is broken, and separated from the egg at various points. B. The first maturation division spindle. C. The first maturation has been completed, and the first polar body lies outside the egg beneath the egg membrane. It appears at the top of the figure and slightly to the right. Just within the egg in the same vicinity is the second maturation spindle, while at about the center of the egg is the sperm head with its aster in front of it. D. The egg and sperm nuclei in the upper left hand part of the egg are fusing, while just beneath the egg membrane is one of the polar bodies. E. The division spindle for the first cleavage. F. The first cleavage is completed and parts of the asters for the second cleavage are indistinctly visible in the two daughter cells.

noted that in this process there is no fusion of the chromosomes. On the contrary, this event, presumably the actual climax of the entire phenomenon, does not occur until the period of synapsis of the germ cells in the new individual, as described above.

The Consequences of Fertilization and Their Importance

We may now consider briefly some of the apparent results of this process and their possible importance. There have been three main consequences of fertilization which have been held to be of vital significance, though as will appear, none of them has proved to be necessarily dependent on this phenomenon. They are as follows:

I. Reproduction. — It has been said that the chief result of fertilization is to bring about reproduction, (a) by restoring the diploid number of chromosomes, and (b) by furnishing or causing to develop a new kinetic division center; i.e., the centrosome. This argument is unsatisfactory for the following reasons:

1. Granting that these events take place in connection with reproduction, the answer is, nevertheless, superficial. For the question immediately arises, why should the egg lose half its chromosomes and its centrosome, thus making fertilization necessary before reproduction can occur?

2. There are numerous cases of both artificial and natural parthenogenesis, showing that neither the extra chromosomes nor the new division center is absolutely necessary.

3. Finally the fact that the union of two cells so frequently precedes reproduction may be explained thus. Let us assume that there is some reason, such as those indicated below, why a mixture of different strains of protoplasm is beneficial. It then follows that in a Metazoan, the only time such a mixture can possibly occur is when the protoplasm of the animals concerned is in the form of single cells; i.e., the germ cells. Then since the animals are in fact Metazoa, the union of the germ cells must eventually be followed by cell division in order that the Metazoan condition may again be reached. Under such circumstances, the multiplication obviously is not proved the result of the fertilization.

II. Rejuvenescence. — It has been widely held that the fusion of different strains of protoplasm which occurs during fertilization is necessary to bring about a revivifying of any given race of animal

or plant. Without this, it is held, cell division will gradually become less frequent, and will finally cease. The chief argument for this view has been furnished by certain experiments on Protozoa. Thus Calkins showed that if Paramœcium was kept from conjugating the strain tended to die out. The best answer to this is the counter experiment of Woodruff, in which, apparently by proper feeding, he has shown conjugation to be unnecessary. There are also certain other facts that seem to controvert this view as to the necessarily vitalizing effect of fertilization. The point already made, however, is enough to prove the theory inadequate.

III. Variation. — A third important result said to be accomplished by fertilization is that it brings about variations on which natural selection may act. It is supposed to do this by mixing the germinal substance (*amphimixis*) so as to make possible new hereditary combinations. This was Weismann's idea. One difficulty with this is that the object of such a mixing of the germ plasm has also been said by Hertwig and others to produce exactly the opposite effect; i.e., to prevent variation. They say that while some new combinations may result, the tendency on the whole is for one extreme to cancel another, and thus keep the race constant. There is no adequate proof for either view, whereas modern genetics throws serious doubt upon both.

Conclusion. — In view of the above facts, the general conclusion as to the function of fertilization may perhaps be stated thus: Neither the process of reproduction nor rejuvenescence is absolutely dependent upon fertilization, while the relation between fertilization and the control of variation is open to much question. Nevertheless, under certain conditions, fertilization does appear to be very intimately connected with both reproduction and rejuvenescence. Now the first of these two processes is obviously always necessary if life is to continue, and the second one, under some circumstances, seems to be equally so. Hence together they may be said to constitute the most important consequences of fertilization of which we are now aware.

EARLY STAGES IN DEVELOPMENT

RELATIVE INFLUENCE OF EGG AND SPERM ON THESE STAGES

In the above discussion of the germ cells it has been stated that despite the great disparity in the cytoplasmic content of the ovum and sperm, their influence upon development is approximately equal. The abundant egg cytoplasm is simply for the purpose of supplying food and material for the nuclear factors to work upon, and varies according to requirements in these respects. The sperm cytoplasm, on the other hand, is only for the purpose of bringing its nucleus to that of the inert egg, and possibly of initiating division. Indeed the very features which characterize the cytoplasm of a particular egg or sperm are presumably determined by genes within the chromosomes, just as are the features which characterize the adult animal.

Nevertheless, it must now be noted that the character of the egg cytoplasm does determine in a rather obviously mechanical way the nature of the early stages in development which we are about to consider. The cytoplasm of the sperm, however, though often strikingly variable in form, is apparently without any such influence. Because of this fact, in the case of most of the animals whose embryology is to be studied, it will be necessary to give a rather full account of the ovum and its development. The various kinds of spermatozoa, on the contrary, will need little further attention.

SEGMENTATION

Subsequent to the first division of the egg which has been indicated, further divisions follow each other, often in relatively rapid succession. The period of these early divisions is termed that of *segmentation* or *cleavage*.

Types of Cleavage. — As has been suggested above, the type of cleavage is largely determined by the nature of the egg cytoplasm, particularly as regards the amount and distribution of the yolk which the latter contains. In a homolecithal egg with relatively little yolk, the cleavage is *total* or *holoblastic*, and approximately equal (Fig. 44). The equality in the size of the cells decreases, however, as the amount of yolk increases. This follows from the fact that where there is much yolk present, it is never equally distributed. Instead it gathers on one
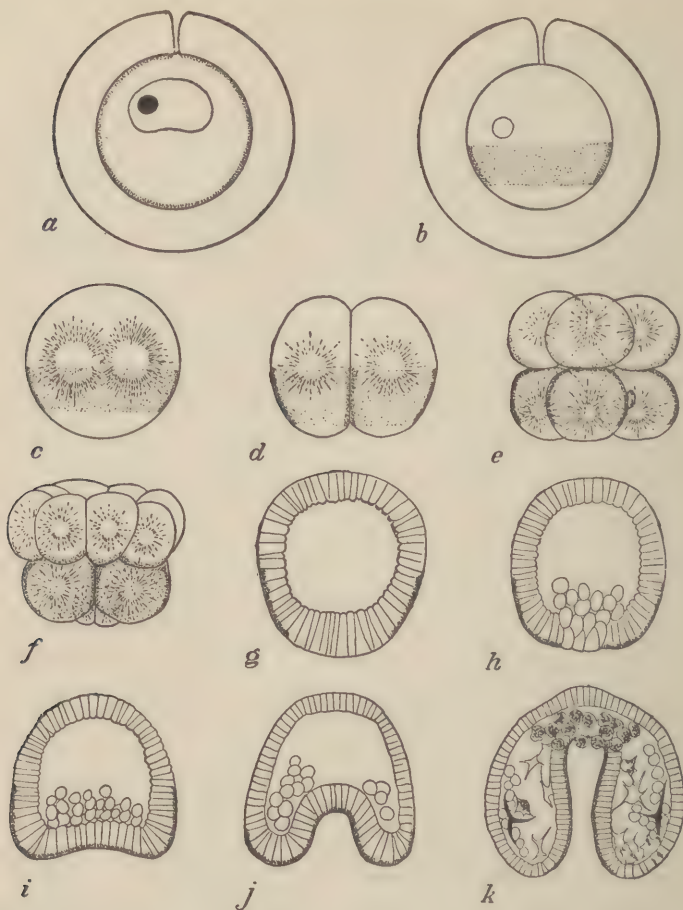
Fig. 44. — Cleavage in the Sea-urchin, *Strongylocentrotus lividus*. From Jenkinson, after Boveri. Animal pole uppermost in all cases.

a. Primary oöcyte surrounded by jelly, and containing large germinal vesicle with nucleolus. Pigment uniformly distributed over surface. *b.* Ovum after formation of polar bodies. Pigment forms a band below the equator. *c, d.* First cleavage. *e.* Eight-cells. Pigment almost wholly in lower quartet (vegetative blastomeres). *f.* Sixteen-cells. The lower quartet has divided latitudinally and unequally, forming four micromeres at the vegetal pole; the upper quartet has divided meridionally forming a plate of eight cells. *g.* Section through blastula. *h.* Later blastula, showing formation of mesenchyme at lower pole. *i, j, k.* Three stages in gastrulation, showing the infolding of the pigmented cells to form the endoderm (archenteron). In *j* the primary mesenchyme is separated into two masses, in each of which a spicule is formed (*k*). In *k* the secondary, or pigmented, mesenchyme is being budded off from the inner end of the archenteron.

side; i.e., the vegetal side, so that the ovum becomes telolecithal. Then since yolk-filled cytoplasm divides with more difficulty than cytoplasm that is free from yolk, inequality of division necessarily results. It is termed simply *unequal cleavage* (Fig. 69). Finally in cases where the amount and density of the yolk is very great, as in many
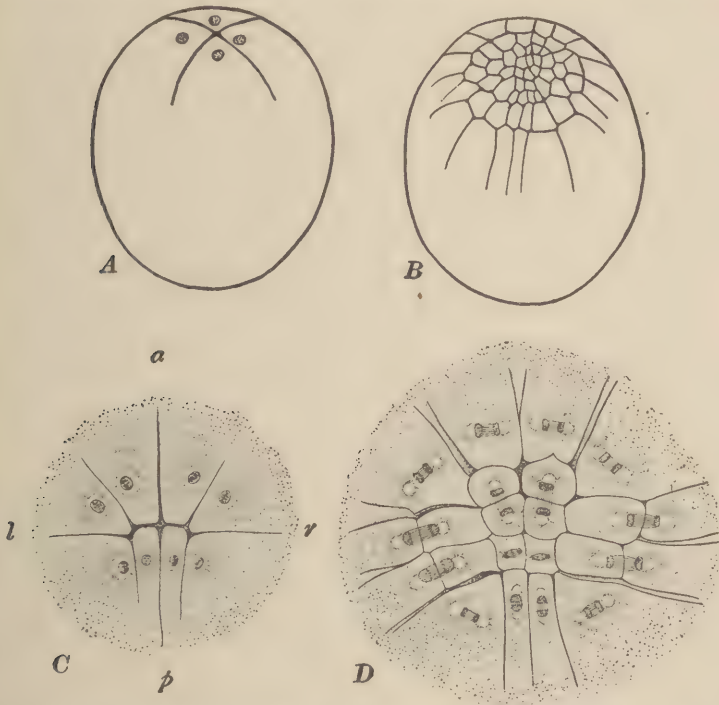
Fig. 45. — Meroblastic cleavage in the Squid, *Loligo pealii*. A, B. Egg viewed obliquely, showing animal pole. x 45. From Kellicott (General Embryology). After Watasé. C, D. Surface views of animal pole, more highly magnified, to show bilateral arrangement of blastomeres. From Wilson, "Cell," after Watasé. A. Four-cell stage. B. About sixty-cells. Cells at the animal pole very small, lowermost cells incomplete, cell walls extending down toward the uncleaved lower pole. C. Eight-cell stage. D. The fifth cleavage (sixteen to thirty-two cells).

a-p. Marks the plane of the first cleavage and the median plane of the organism. *l-r*. Marks the second cleavage, and the transverse plane of the organism.

Fishes and Birds, that part of the egg which contains it does not cleave at all, or only very slightly. In such eggs, as already noted, the yolk-free cytoplasm exists only as a small accumulation at the animal pole of the egg, called the *blastodisc*. It is then chiefly this disc which

divides; after division it is called the *blastoderm*. Cleavage of this type is known as *meroblastic*, or *discoidal* (Figs. 45, 46).

The Blastula. — After cleavage has continued for a time in an egg of the homolecithal type a hollow sphere of cells results, with a cavity at or near its center (Figs. 47, *A*; 44, *g*). Such a sphere is called a *blastula,* and the cavity at its center is termed the *segmentation cavity,* or *blastocœl.* In eggs of the markedly telolecithal type there also
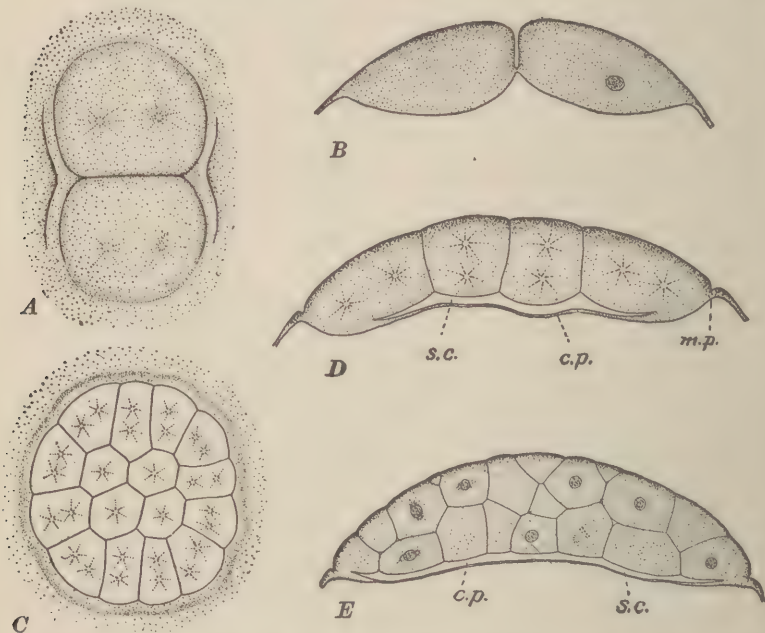
Fig. 46. — Cleavage in the Sea-bass, *Serranus atrarius.* From H. V. Wilson. A. Surface view of blastoderm in two-cell stage. B. Vertical section through four-cell stage. C. Surface view of blastoderm of sixteen cells. D. Vertical section through sixteen-cell stage. E. Vertical section through late cleavage stage.
c.p. Central periblast. *m.p.* Marginal periblast. *s.c.* Segmentation cavity (blastocœl).

exists at the completion of cleavage a sphere, but in this case, as has been noted, the greater part of it consists of undivided yolk. It is nevertheless termed a blastula, and the segmentation cavity will lie at the animal pole between the largely unsegmented yolk mass and the blastoderm (Figs. 48, *A*; 46, *D*, *E*). Although cell division continues, the cleavage stage may be said to end when the blastula condition has been reached.

GASTRULATION

Gastrulation is the process by which two different types of cells become definitely set apart in the form of layers, the outer layer being termed *ectoderm* and the inner layer *endoderm*. Also in connection with this there is produced a cavity more or less distinct from the segmentation cavity, termed the *archenteron,* while the embryo as a whole is now known as a *gastrula*. The new cavity or archenteron is the *primitive gut*. At first it may be only partially lined by the endoderm; eventually, however, in the process of its transformation into the real or *definitive gut* or *enteron* it always becomes entirely so lined. Gastrulation having been thus defined, it now becomes necessary to indicate briefly and in a general way the processes through which it may occur. For the sake of clearness and convenience these processes will be described separately, though it should be noted that in the majority of actual cases two and often more of them take place together.

Invagination. — Probably the simplest method of gastrulation is by *invagination,* a method which is sometimes spoken of as being typical. As a matter of fact, however, the accomplishment of gastrulation by this means alone is rather exceptional even among the Invertebrates, and among the Vertebrates it never occurs to the exclusion of other methods. Indeed within the latter phylum it is found in a relatively unmodified form only among a few of the very lowest members of the group. In all the higher animals it is very largely altered and augmented by other means, and in many instances appears not to be present at all. In its simplest and most unmodified condition, however, it may be described thus:

Let the blastula be thought of as a hollow sphere, one hemisphere of which is to be regarded as the animal half and the other hemisphere as the vegetal half, while the cavity within the sphere is the blastocœl (Fig. 47, *A ;* 44, *g*). Now, imagine the vegetal half to be pushed in or invaginated until it almost touches the animal half opposite to it. The sphere has thus become a gastrula. The original blastocœl has been virtually obliterated and a new cavity has been formed by the invagination. This is the archenteron, and it is lined by the original vegetal cells which may now be termed endoderm (Figs. 47; 44, *k*). The cells which constitute the animal hemisphere, on the other hand, are henceforth called ectoderm. The opening of the

archenteric cavity to the exterior is then in this case the *blastopore*, and the rim of this opening the *lip of the blastopore*. It must be immediately stated, however, that only in eggs of a relatively yolk-less character, is the blastopore thus a wide-open orifice. As the amount of yolk increases it tends to fill both the archenteron and its opening more and more, until in eggs of the extremely telolecithal type there is very little left of the archenteron as a cavity or of the blasto-pore as an opening. Thus in eggs of this sort the boundary of the blastopore; i.e., the blastoporal lip, is really the edge of the blastoderm. To cover all cases, therefore, it is perhaps better to describe the lip
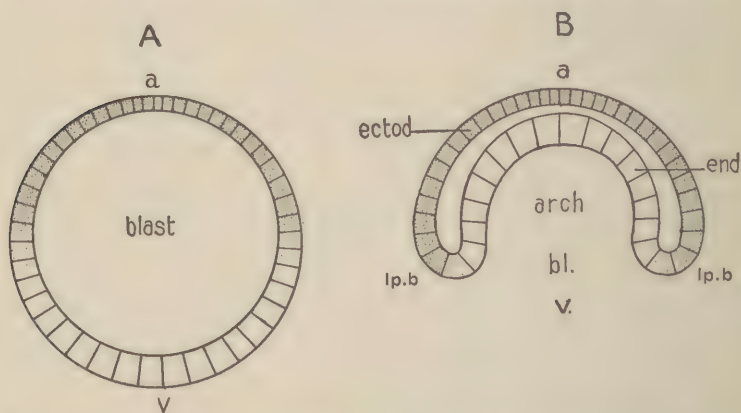
Fig. 47. — Diagrammatic representation of gastrulation by invagination.   A. Ideal meridional section of a blastula.   B. Ideal meridional section of a gastrula.   *a.* Animal pole.   *arch.* Archenteron.   *blast.* Blastocœl.   *bl.* Blastopore.   *ectod.* Ectoderm.   *end.* Endoderm.   *lp.b.* The lip of the blastopore or germ ring.   *v.* Vegetal pole.   The cells at the vegetal pole are usually larger because they contain more yolk.

of the blastopore as the line of undifferentiated tissue where ectoderm and endoderm merge with one another. This description it will be found applies to the edge of the blastoderm as well as to the rim of a blastopore which possesses a wide opening. It may now be added that the lip of the blastopore is also often called by another name; i.e., the *germ ring*. The reason for this is the fact that it was once thought that a very large portion of each side of the embryo always originated from this ring in a manner to be described below (see con-crescence). Though later investigation has shown that this is often true only to a limited degree, still the region thus named is frequently characterized by especially rapid cell division, and is known in many

cases to furnish much of the material for the dorso-median embryonic axis.

Involution. — A second process of gastrulation may be described as involution or inflection. It is very common among the Vertebrates, and, within this group at least, it probably always accompanies any invagination which may occur. In many cases also it appears to be the chief factor involved, particularly among those forms which arise
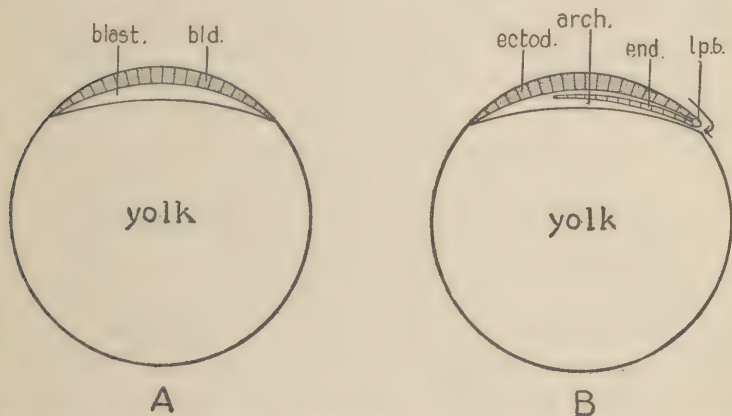
Fig. 48. — Diagrammatic representation of gastrulation by involution in the case of an egg with a large yolk mass which does not segment. A. Ideal meridional section of a blastula. B. Ideal meridional section of a partially completed gastrula, bisecting the dorsal blastoporal lip. *arch.* Archenteron. *blast.* Blastocoel. *bld.* Blastoderm. *ectod.* Ectoderm. *end.* Endoderm. *lp.b.* The lip of the blastopore. The arrow points to the blastopore, and indicates the movement of involution.

from a telolecithal egg. For this reason it is most convenient to describe the process as it occurs in an egg of this type.

In such eggs it has been noted that the yolk usually does not segment at all, and that in correlation with this the segmentation cavity will be greatly reduced (Fig. 48, *A*). Under such conditions it is evident that gastrulation can not occur by simple invagination because the mass of yolk filling the center of the blastula will not permit it. What does happen, therefore, is this: At some point on the edge of the blastoderm (see above), the dividing cells, instead of extending out over the unsegmented yolk, begin to be turned over the blastodermal rim; i.e., *involuted* into the segmentation cavity. These inturned cells then constitute the endoderm, while those which remain without are ectoderm (Fig. 48, *B*). According to definition, therefore,

the edge of the rim, in this case the edge of the blastoderm, is the blastoporal lip or germ ring, while the movement over this lip is designated as *involution*. As suggested above, however, this process is not confined to animals with a large yolk mass, and it is to be clearly understood, therefore, that the only essential feature concerned is the passage of cells over the lip. It is this movement, which, as stated, comprises involution, and this remains true whether the active cells be arranged in the form of a blastoderm or otherwise. In some instances where the yolk mass is very great, as in Birds and many Fishes, the movement is accompanied by virtually no invagination. In
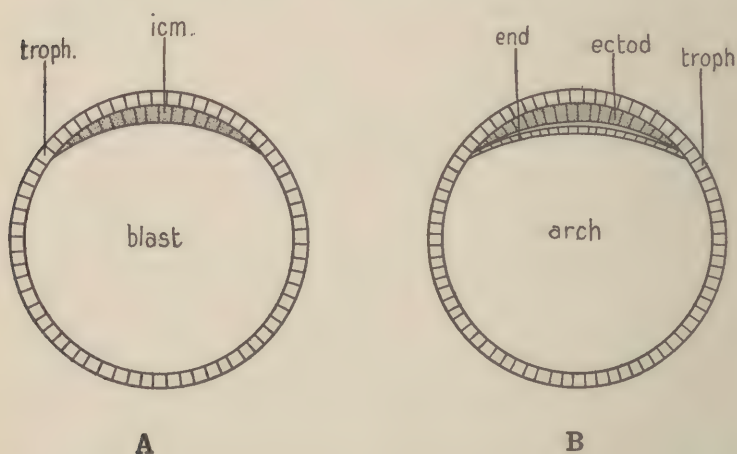
Fig. 49. — Diagrammatic representation of gastrulation by delamination. A. Ideal meridional section of a blastula, or as it is called in Mammals, the blastocyst. B. Ideal meridional section of a gastrula. *arch*. Archenteron, as yet only partially lined by endoderm and lacking a blastopore. *blast*. Blastocœl. *ectod*. Ectoderm. *end*. Endoderm. *icm*. Inner cell mass, virtually homologous with the blastoderm of blastulas with much yolk. *troph*. Trophoblast, an embryonic layer peculiar to Mammals, (see chapter XIV).

others (Amphioxus and Amphibians), the later process also takes place to a greater or less extent. In any event the inflection or involution is most active in that portion of the blastoporal lip which eventually proves to be dorsal. The degree and character of its occcurence in other parts of the lip vary considerably in different animals, and can best be indicated later in specific cases.

Delamination. — A third process by which gastrulation may occur is that of delamination, and so far as Vertebrates are concerned it is

found in its most clearly defined condition among Mammals. According to Brachet, however, it is also involved to a certain extent in the development of the Amphibians. Within the former animals the phenomenon appears to be both simple and exclusive of any other processes, and may be described, in such instances, as follows. Starting with a hollow blastula, endodermal cells are proliferated from the ventral side of a cellular mass (*inner cell mass*) adherent to the roof of the blastocœl (Fig. 49, *A*). These cells are then split off or delaminated from the region which gave rise to them, and thus constitute a separate layer, the endoderm (Fig. 49, *B*). Further proliferation of the cells of this layer then follows, and there thus arises an archenteron, temporarily lacking a blastopore, and with an interior cavity which was once the blastocœl. The mass which gave rise to the second layer may thereafter be termed ectoderm. A further and more detailed discussion of this method of gastrulation will be found in Chapter XIV. Likewise the somewhat complicated and subsidiary part which it appears to occupy in the case of Amphibians will be taken up under the development of the Frog. Regardless of entangling details, however, it should be remembered that the essential feature of the process is the origin of the two primary layers by the splitting off of one from the other, and this remains true in whatever situation it may occur.

Accessory Processes. — Save in those cases where delamination is the sole factor in gastrulation, two other processes are probably always to some extent involved, and in most instances are of considerable prominence. As will presently appear, however, these movements, at least among Chordates, are not strictly a part of gastrulation proper; i.e., they do not actually differentiate endoderm from ectoderm, though they aid in the extension and disposition of both these layers. Hence they may be more correctly regarded as accompanying or accessory activities.

1. Epiboly. — This is the first of these accessory movements, and occurs most typically in the development of eggs possessing abundant yolk; e.g., those of Fishes and Amphibians. It merely involves the gradual growth of the blastoporal lip over the yolk, or the yolk-filled vegetal cells. It may be roughly pictured (Fig. 50) by imagining a solid sphere, the yolk, over which a rubber cap, the blastoderm, is being stretched, the rim of the cap representing of course the lip of the blastopore. The movement, however, is not due ap-

parently to any actual process of stretching, but rather to active cell division in the overgrowing layers of ectoderm and endoderm. As previously suggested, this activity is thought to be most intense in the region of the lip itself; i.e., the germ ring. At all events the result of such a process will obviously be eventually to enclose the yolk as in a sac (the *yolk sac*); the completion of this process necessarily involves also the closure of the blastopore (Fig. 50).

II. Concrescence. — This is the second activity which may be regarded as an accompaniment of gastrulation, though not perhaps as
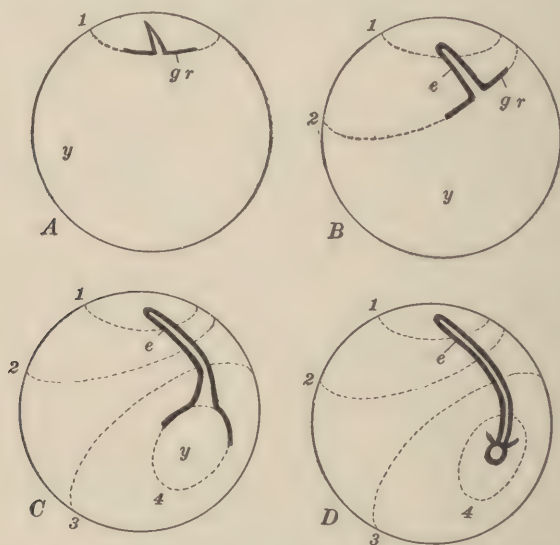
Fig. 50. — Diagrams illustrating four stages in the formation of the Teleost embryo, (having an extremely telolecithal egg) and the growth of the germ ring or lip of the blastopore around the yolk mass (epiboly). From Kellicott (General Embryology). After O. Hertwig. *e.* Embryo. *gr.* Posterior margin of the germ ring. *y.* Yolk mass. **1, 2, 3, 4,** successive positions occupied by the germ ring as it advances over the yolk.

invariably so as is the first. It may be described thus. As the process of epiboly goes forward there always results, as noted, a gradual drawing together of the blastoporal lips, so that the size of the blastopore itself is diminished. Furthermore, in the course of this procedure there is not, contrary to what might be expected, any noticeable puckering or thickening of the lips as their circumference decreases. This fact may be readily accounted for by assuming that much of the material which they contain is required to furnish the ectoderm and

endoderm which they are leaving behind them. Aside from this, however, there has been demonstrated in many cases another source for the consumption of at least part of the surplus substance of the germ ring. Thus as gastrulation proceeds it would appear that the two sides of the germ ring are flowing together at a certain point upon the margin of the blastoderm, this movement being aptly designated as *confluence* or *concrescence* (Fig. 51). In consequence of this process, moreover, there soon arises a line of concresced material, lying along what proves later to be the mid-dorsal line of the embryo. It is in this manner, therefore, that in many instances, as suggested above, the two halves of the blastoporal lip furnish much of the material for the axial structures of the animal. Finally, it should be pointed out that the essential feature concerned in this process is the flowing together of the sides of the blastopore. Thus, like involution, this movement can and does occur, in eggs with relatively little yolk as well as in those which are telolecithal.
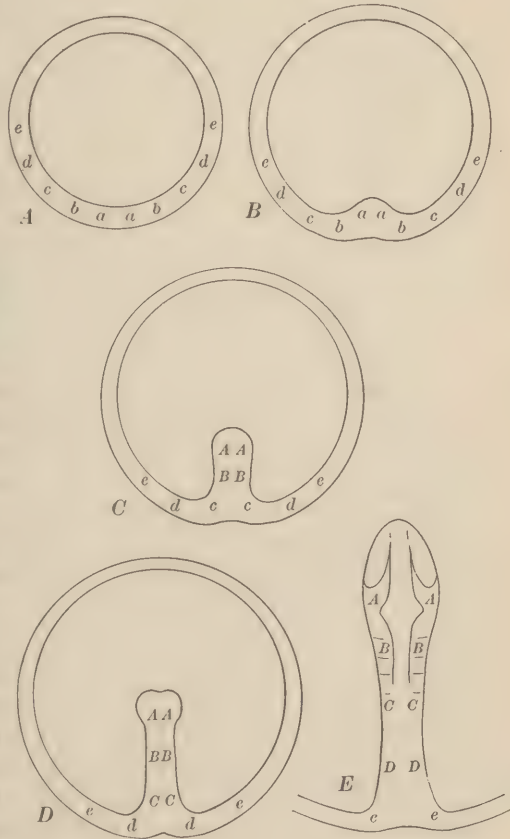
Fig. 51. — Diagram of the formation of an embryo by confluence (concrescence). From Kellicott (General Embryology). A. Germ ring before formation of the embryo is indicated. The letters *a–e* represent symmetrical portions of the germ ring. B. Beginning of confluence. C. Embryo forming. *AA*, *BB*, represent regions of the embryo formed out of the materials of the germ ring at *aa*, *bb*. D, E. Later stages in the formation of the embryo. The germ ring regions cc and dd, have been differentiated into the embryonic regions, *CC*, *DD*.
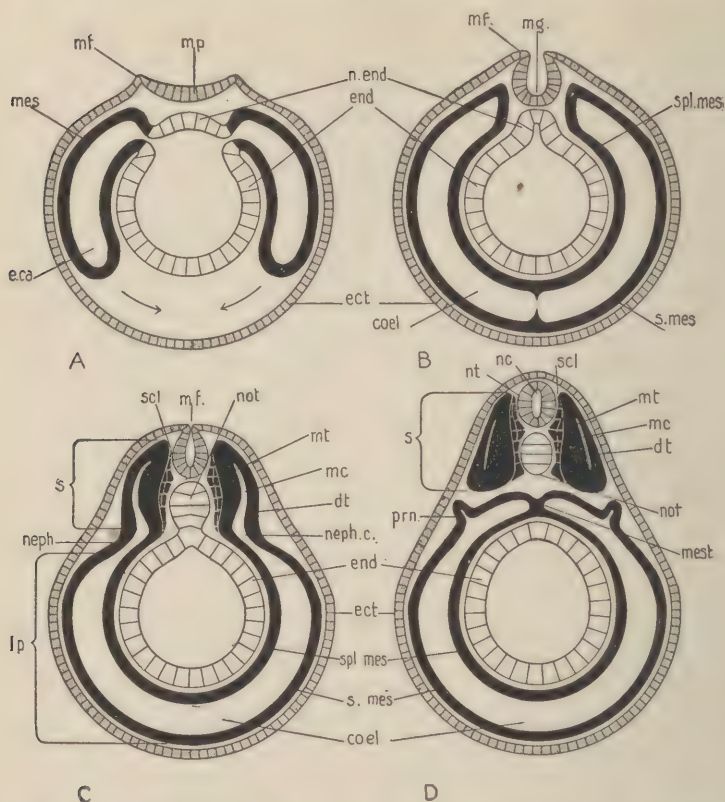
Fig. 52. — A diagram of the origin and early differentiation of the mesoderm, and of the notochord and nerve cord in a generalized Vertebrate.

A. The mesoderm is arising by means of enterocœlic pouches which are pushing out from the archenteron and are not yet separated from it. B. The enterocœlic pouches have separated from the archenteron, their walls forming the splanchnopleure and somatopleure, and their cavities the cœlom. The notochord is beginning to develop and the medullary folds are approaching each other. C. The regions of the vertebral plates, which are divided transversely into somites, the nephrotomes and the lateral plates are marked out, and the various parts of the somites are distinguishable. D. The closing of the neural tube or nerve cord is completed. The somites are further developed and the myocœl is nearly obliterated. The notochord is separated from the archenteron and the mesentery has formed. The pronephros or embryonic kidney is developing from the nephrotome.

coel. Cœlom.   *dt*. Dermatome.   *ect*. Ectoderm.   *e.ca*. Enterocœlic cavity.   *end*. Endoderm.   *lp*. Region of the lateral plate.   *mc*. Myocœl.   *mf*. Medullary folds.   *mg*. Medullary groove.   *mp*. Medullary plate.   *mes*. Mesoderm originating in this case by the enterocœlic method.   *mest*. Mesentery.   *mt*. Myotome.   *nc*. Neural canal.   *neph*. Region of the nephrotome.   *neph.c*. Region of the nephrocœl.   *not*. Notochord.   *nt*. Neural tube or nerve cord.   *prn*. Rudiment of the pronephros or embryonic kidney.   *s*. Region of segmental or vertebral plate (somites).   *scl*. Sclerotome.   *s.mes*. Somatic mesoderm.   *spl.mes*. Splanchnic mesoderm.

FORMATION OF MESODERM AND CŒLOM

All animals whose tissues are formed from three fundamental cell layers are said to be *triblastic*. The Chordates belong to this group and therefore possess a third embryonic layer, the *mesoderm,* which arises between the first two. In point of time the origin of this layer may more or less coincide with the differentiation of the ectoderm and endoderm, though the process is essentially distinct from gastrulation. Its source may be either of the two layers already developed, or in some cases both. More typically, however, it arises from the endoderm. There are three chief methods by which the mesoderm is developed, the first of which is rather intimately connected with the origin of the cœlom. In the remaining two, these processes, as we shall see, are not quite so closely correlated.

I. The Enterocœlic Method. — This method, though common among certain Invertebrates, occurs in connection with only a few of the lowest members of the Chordate phylum. In its general aspects it may be described thus. Along each side of the archenteron in its doral region there arise a series of hollow outpushings or evaginations which grow outward between the endoderm and ectoderm. The walls of the sacs thus formed then constitute the mesoderm, while the cavities of these sacs, because of their archenteric source, are called *enterocœls,* or *archenteric pouches* (Fig. 52, *A*). Following this, the mesodermal sacs become cut off from the archenteron, while at the same time they continue to enlarge until those upon opposite sides meet one another ventrally (Fig. 52, *B*). Likewise successive sacs upon the same side also come in contact. The walls separating the major portions of the enterocœls then disappear, so that a single cavity, the *cœlom,* entirely encircles the enteric canal (former archenteron) except along its mid-dorsal line (Fig. 52, *C*). The mesodermal lining of this cavity in contact with the canal is termed the *splanchnopleure* or *splanchnic* mesoderm, while that next to the ectoderm is the *somatopleure* or *somatic* mesoderm. Just above the canal the layers of splanchnopleure from either side eventually unite into a sheet which virtually suspends the enteron from the dorsal body wall and is known as a *mesentery* (Fig. 52, *D*). Along the dorsal border of this mesentery the two layers of which it is composed again separate and pass in opposite directions to line the cœlom as the somatopleure already indicated. From the above account it will be noted that this

is one of the instances in which the origin of the mesoderm is clearly entirely endodermal.

It remains to be observed that, despite the rarity of this method of mesoderm formation among the Chordates, it is regarded nevertheless as of considerable zoölogical interest. The reason for this is the fact, already suggested, that it is found abundantly in some of the large Invertebrate groups (e.g., the Echinoderms and Prosopygia), and is then repeated among the lowest Chordata. This is significant because such repetition in these members of the Chordate phylum is suggestive in helping to determine from which class of Invertebrates the Vertebrate group arose.

II. **The Method of Delamination.** — The production of a cell layer by a method whose essential feature was a splitting off or delamination of cells has already been noted in connection with the origin of endoderm. It now remains to be stated that a similar process is quite frequent among Vertebrates with respect to the generation of mesoderm. In those cases where it occurs, the critical element merely involves, as before, a separation of cells from a pre-existing layer to form a new one. Here, however, the layer from which the cells are derived is the endoderm, while the new layer to which they give rise is the mesoderm. The latter in turn is itself then split into two layers, the somatopleure and splanchnopleure, while the space which arises between them is the cœlom. The relations of the somatic and splanchnic linings of this cavity to the body wall and to the enteron and the subsequent development of other parts are the same as in Method I.

III. **The Method of Proliferation.** — This, a third method, prevails chiefly among the highest Vertebrates. Here the mesoderm arises by the budding off of cells from a particular region, often apparently ectodermal, and situated along the mid-dorsal line. These cells then spread out between the ectoderm and the endoderm, and there is thus formed a third layer similar to that produced in the previous example by delamination. From this layer the formation of the somatopleure, splanchnopleure, and cœlom then follows as in Method II, while the later development is again similar to that occurring in the other two cases.

It is to be noted that it is only in this last instance that the ectoderm appears possibly to function as an immediate source of the mesoderm.

The Sources of the Tissues

The three embryonic cell layers having thus been defined and their origin described, the subject may be concluded by indicating in a general way the tissues to which each cell layer eventually gives rise.

1. **The ectoderm** produces the epidermal part of the skin, including cutaneous glands, hair, feathers, nails, hoofs, and one type of horns and scales. It also gives rise to parts of the eye, the external and internal ear, and the lining of the anus and oral cavities, including the enamel of the teeth. It is the origin of the entire nervous system and a few muscles.

2. **The mesoderm** gives rise to most muscles, as well as to adipose tissue and all other varieties of connective tissue including the dermis, certain types of scales and horns and the main portion (dentine) of the teeth. It also produces the skeletal system, the blood vascular system, and the greater part of the urino-genital system. It forms the cœlomic epithelium, mesenteries, and outer layers of the alimentary tract.

3. **The endoderm** produces the lining of the alimentary tract and the epithelial parts of all the organs which arise as outgrowths from it; i.e., the respiratory system, the thyroid and thymus glands, the liver, and the pancreas. It also lines the auditory tube and middle ear, and forms a small part of the urino-genital system.

The Notochord

A characteristic feature of the embryos of all true Chordates is a rod of vacuolated tissue lying along the mid-dorsal line just above the gut. It is termed the *notochord,* and makes its appearance at about the same time at which the mesoderm starts to develop, or in some instances somewhat later. It is clearly derived in many cases from the dorsal wall of the archenteron; i.e., it is endodermal (Fig. 52, *B, C, D*). In some instances, however; e.g., in Birds and Mammals, the origin of the notochord is apparently partially or entirely ectodermal. The position which the structure occupies is obviously that which is taken by the vertebrae of the higher adult Chordates; e.g., the genuine Vertebrates. As will appear the bony structures which thus replace the notochord in the latter animals arise from certain of

the mesodermal tissues which surround it, while the notochord itself is gradually absorbed.

The Lateral Plates, the Somites, and the Nephrotomes

Among all the Chordates, except in the case of a few of the most primitive members of the group, there accompanies or immediately follows the development of the cœlom, certain other fundamental differentiations of the mesoderm. These differentiations result in the formation of three major divisions of this substance, whose origin and character may be described in a general way as follows:

I.   The Lateral Plates. — It has already been suggested that the main portion of the mesoderm upon each side of the animal gives origin to the cœlom and its lining. It remains to state that each of these portions is frequently known as a *lateral plate*.

II. The Vertebral Plates. — The mesoderm which is not involved in the production of the lateral plates, nevertheless remains connected with them for a time, lying dorsally along either side of the notochord and nerve cord in the form of a relatively narrow band, a *vertebral* or *segmental plate* (Fig. 52, *C*). The major portion of each band (i.e., all of it, save a narrow strip connecting it ventrally with the respective lateral plate) then thickens somewhat, and soon begins to be transversely divided into a series of block-like masses termed *somites*. The more anterior members of the series usually appear first, and each one as it is formed proceeds to give rise to three fundamental elements: the *dermatome*, the *myotome*, and the *sclerotome* (Fig. 52, *C*, *D*). Of these elements the relatively thin dermatomes lie next to the ectoderm, and are concerned chiefly in the production of the deeper layer of the skin; i.e., the dermis. The thicker myotomes come beneath and median to the dermatomes and give rise to the bulk of the voluntary muscles, while the sclerotomes, arising as proliferations of scattered cells, are nearest the notochord and produce the skeletogenous tissue of the axial skeleton.

It may be further remarked that in many instances at this period a small portion of the cœlomic space extends up into each somite between the dermatome and myotome, and is there known as a *myocœl*. Like the connection between the somites and the lateral plates, however, it is of only temporary duration.

In Amphioxus, one of the very primitive Chordates referred to above, it should be noted that the term somite as used in the early

history of this animal is somewhat more inclusive than in the fore-going description. Thus in this instance these bodies when newly formed, comprise not only the elements of the dermatomes, myotomes, and sclerotomes, but likewise those of the lateral plates. It may finally be added that since there are no bones in Amphioxus, the sclerotomes give rise only to connective tissue.

III. The Nephrotomes. — It will be recalled that of each band of mesoderm lying between the lateral plate and the notochord, the major dorsal portion goes to form the somites. The remaining narrow strip, which for a time connects these bodies with the correspond-ing lateral plate, is then designated as the *nephrotome* or *intermediate cell mass,* while its cavity, temporarily uniting the main cœlom and the myocœls, is the *nephrocœl* (Fig. 52, *D*). The nephrotomes later contribute chiefly to the formation of the excretory organs.

In Amphioxus and the other primitive Chordata no nephrotome exists, and the excretory organs are therefore of an entirely different character and origin.

THE DORSAL NERVE CORD

The final fundamental feature of Chordate anatomy which appears in connection with these very early embryonic stages is the *dorsal nerve cord* or *neural tube.* The latter term is used not only because it indicates a characteristic of this structure which is peculiar to Chordates, but also because it suggests the method of its development, which is likewise peculiar to this group. This method is as follows.

During or shortly following the processes of gastrulation a broad strip of ectoderm along the future dorsal side of the animal becomes thickened, this thickened area being termed the *medullary* or *neural plate* (Fig. 52, *A*). The median portion of this plate then becomes depressed slightly to form a groove, the *medullary* or *neural groove,* while the sides are correspondingly elevated as the *medullary* or *neural folds* (Fig. 52, *B*). These folds gradually grow toward one another until their crests meet and fuse, and there is thus developed a tube, which presently becomes entirely separated from the ectoderm above it (Fig. 52, *C*, *D*). This is the rudiment of the nerve cord or neural tube, while the canal which traverses its center is the *neural canal* or *neurocœl.*[3] At its anterior end this canal opens to the ex-terior for a time through a small aperture, the *neuropore.* At the

[3] This method of nerve cord formation is, as noted, characteristic of most Vertebrates, but is modified somewhat in the case of the Lampreys and many

posterior end, on the contrary, the fusion of the medullary folds eliminates the external opening (except in some Sauropsids and Mammals) at an early stage, but preserves an internal passage-way as follows. Instead of stopping dorsal or anterior to the nearly closed blastopore, the above folds extend slightly downward or backward upon either side of it. They then fuse above the latter orifice in such a way that through it, for a considerable time, the neurocœl communicates with the enteric cavity. The short bent portion of the neurocœl in this particular region, together with the remains of the blastopore, is then known as the *neurenteric canal* (Fig. 61).

The process thus described has already been indicated as characteristic of all true Chordates, and as regards all fundamental points this is true. It should be stated, however, that once more in the case of Amphioxus certain minor variations occur. These will be considered in connection with the development of that animal.

REFERENCES TO LITERATURE

Abbreviations for the names of periodical publications referred to in the literature cited at the ends of chapters are as follows:

Am. Jour. Anat. = *American Journal of Anatomy*, Baltimore and Philadelphia.
Am. Jour. Physiol. = *American Journal of Physiology*, Boston.
Anat. Anz. = *Anatomischer Anzeiger*, Jena.
Anat. Hefte = *Anatomische Hefte*, Wiesbaden.
Anat. Rec. = *Anatomical Record*, Philadelphia.
Arbeit Zool. Inst. Wien. = *Arbeiten aus den zoologischen Institute zu Wien*.
Arch. Anat. u. Entw. = *Archiv für anatomie und Entwickelungsgechichte*, Leipzig. (Same as Arch. Anat. u. Physiol.)
Arch. Anat. u. Physiol. = *Archiv für Anatomie und Physiologie*, Leipzig.
Arch. Biol. = *Archives de Biologie*, Leipzig and Paris.
Arch. d'Anat. Micr. = *Archives d'Anatomie Microscopique*, Paris.
Arch. Entw.-mech. = *Archiv für Entwickelungsmechanik der Organismen*, Leipzig.
Arch. mikr. Anat. = *Archiv für mikroskopische Anatomie und Entwickelungsgeschichte*, Bonn.
Arch. Zellf. = *Archiv für Zellforschung*, Leipzig.
Arch. Zool. Exp. = *Archives de Zoologie expérimentale et générale*, Paris.
Biol. Bull. = *Biological Bulletin*, Wood's Hole, Mass.

of the Teleost Fishes. Thus in these animals no groove is formed in the thickening medullary plate. Instead the latter simply presses downward beneath the surface as a solid cord of tissue. The neural canal then arises later within this cord by the separation or disintegration of the cells along its axis. (Fig. 129).

Biol. Centr. = *Biologisches Centralblatt*, Leipzig.
B. M. C. Z. Harvard = *Bulletin of the Museum of Comparative Zoology at Harvard College*, Cambridge, Mass.
Bull. Soc. Imper. Moscou = *Bulletins de la Société Impériale de Natural — de Moscou.*
C. R. Soc. Biol. Paris = *Comptes rendus des séances et memoires de la Société de Biologie*, Paris.
Ergeb. Anat. u. Entw. = *Ergebnisse der Anatomie und Entwickelungs-geschichte*, Wiesbaden.
Festsch. f. Gegenbaur. = *Festschrift für* Gegenbaur, Leipzig.
Intern. Monatsschr. = *Internationale Monatsschrift für Anatomie und Physiologie*, Leipzig.
Jena Zeitschr. = *Jenaische Zeitschrift für haturwissenschaft*, Jena.
Jour. Anat. Physiol. = *Journal of Anatomy and Physiology*, London.
Jour. Exp. Zool. = *Journal of Experimental Zoology*, Baltimore and Philadelphia.
Jour. Morph. = *Journal of Morphology*, Boston and Philadelphia.
Jour. Univ. Tokyo = *Journal of the College of Science, Imperial University of Tokyo.*
Kgl. Svensk. Vet. Handl. = *Kongliga Svenska Vetenskapsakademie, Abhand-lugen aus der Naturlehre*, Leipzig.
Mem. Acad. Imper. St. P. = *Memoires de l'Academie Impériale de St. Petersbourg.*
Mem. Acad. roy. Belg. = *Memoires de l'Academie royale de Belgique.*
Mem. Boston Soc. Nat. Hist. = *Memoirs of the Boston Society of Natural History.*
Mem. N. Y. Acad. Sci. = *Memoirs of the New York Academy of Sciences.*
Mitt. Zool. Stat. Neapel = *Mitteilugen aus der zoologischen Station zu Neapel*, Berlin.
Morph. Jahrb. = *Morphologisches Jahrbuch*, Leipzig.
Phil. Trans. Roy. Soc. = *Philosophical Transactions of the Royal Society of London.*
Proc. Am. Phil. Soc. = *Proceedings of the American Philosophical Society*, Philadelphia.
Proc. Internat. Cong. Zool. Cambridge = *Proceedings of the International Congress of Zoologists*, Cambridge.
Proc. Zool. Soc. = *Proceedings of the Zoological Society of London.*
Q. J. M. S. = *Quarterly Journal of Microscopical Science*, London.
Tijd. Nederl. Dierk. Ver. = *Nederlandsche dierkundige Vereeniging, Tijdschrift*, Leyden.
Trans. Am. Phil. Soc. = *Transactions of the American Philosophical Society*, Philadelphia.
Verh. d. Anat. Gesell. = *Verhandlungen der Anatomishen Gesellschaft.*
Verhand. Kon. Akad. Wetensch. = *Verhandelingen koninklijke Akademie van Wetenschappen*, Amsterdam.
Zeit. Anat. Entw. = *Zeitschrift für Anatomie und Entwickelungsgeschichte*, Leipzig.
Zool. Jahrb. = *Zoölogische Jahrbücher*, Jena.

CHAPTERS I AND II

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THE EARLY DEVELOPMENT OF AMPHIOXUS

The early stages in the development of Amphioxus (*Branchiostoma lanceolatum*) are taken up because in this form these stages are thought to be as nearly primitive as those occurring in any other Chordate. This applies particularly to the method of segmentation, gastrulation, and formation of the mesoderm and cœlom. Indeed the general resemblance of these processes to what occurs among Invertebrates, such as the Echinoderms, is so marked that their primitive character in Amphioxus can hardly be doubted.

In the subsequent description it is assumed that the student has in mind a fair knowledge of the anatomy of this animal. The account of the processes involved is based upon that of Cerfontaine.
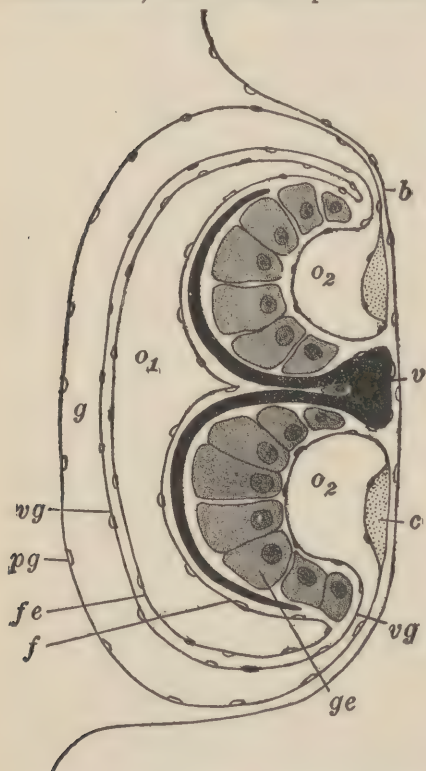
Fig. 53. — Diagram of a section through the gonad of Amphioxus. From Kellicott (Chordate Development). After Cerfontaine.

b. Peribranchial (atrial) epithelium. *c*. Cicatrix. *f*. True follicular epithelium. *fe*. External layer of follicular epithelium. *g*. Gonocœl. *ge*. Germinal epithelium. o_1. Primary ovarian cavity. o_2. Secondary ovarian cavity. *pg*. Parietal layer of gonocœl. *v*. Cardinal vein. *vg*. Visceral layer of gonocœl.

THE REPRODUCTIVE ORGANS

THE OVARY

The ovaries are developed in each myocœl (Fig. 62) on both sides of the body from the

tenth to the thirty-fifth or thirty-sixth somite inclusive. Each originates
as a proliferation of cells on the antero-ventral wall of the myocœl.
This proliferation then pushes forward as a small bud, covered by
the portion of the myocœlic wall from which it arose. The bud of
germ cells with its covering thus comes to project sac-like into the
myocœl anterior to the one in which the proliferation started. The
neck of the sac then forms a short stalk connecting it with the pos-
terior myocœlic wall of the cavity into which the evagination has
occured. Thus in these animals each egg is not surrounded by its
individual follicle, but is attached to the wall of the above sac, which
acts as a general follicle for all the ova within it. As development
proceeds, the most ventral part of each myocœl which contains the
gonad is cut off from the part above as the *gonocœl*. By the time
a batch of ova is ripe, however, which occurs for the first time in
animals about two centimeters in length, each ovary has grown so
that it virtually obliterates all cœlomic spaces surrounding it (Fig.
53). These eggs are then extruded (see below), while the ovary
during the process almost disappears. It then develops anew in
preparation for the next breeding season.

THE TESTIS

The development of the testes in Amphioxus is not so well known,
but it appears to be similar in a general way to that of the ovary.
The products are discharged to the outside as are the eggs.

THE HISTORY OF THE OVUM TO GASTRULATION

OÖGENESIS

Multiplication and Growth. — After passing through a typical
oögonial or multiplication stage the cells cease dividing and enter upon
a period of growth. During this period the nucleus passes through
the last processes prior to maturation, while deutoplasm appears
throughout the greater part of the cytoplasm. Inasmuch as this is a
comparatively yolk-free egg the latter substance does not become very
dense. It does become just abundant enough, however, so that the
yolkless portions are clearly distinguishable. The latter eventually
consist of (1) a thin vacuolated area lying everywhere just beneath the
surface (Fig. 54, *A*), and (2) a somewhat conical region situated to-

ward one side of the egg with the apex outward (Fig. 55). The side thus occupied is therefore the animal pole within whose cone of clear cytoplasm lies the nucleus, while the opposite side is the vegetal pole. Thus the yolk-free area is eccentric and the egg is really telo-
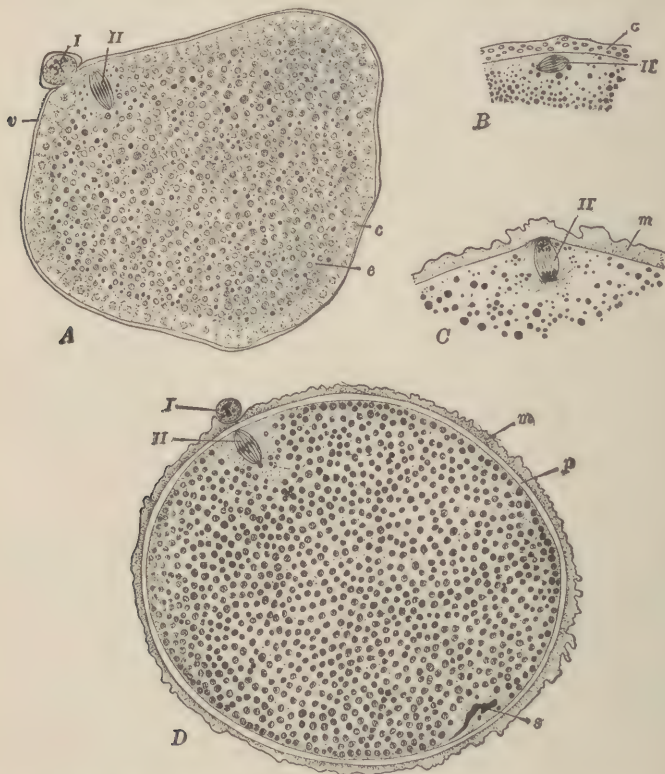
Fig. 54. — The egg of *Amphioxus*. From Kellicott (Chordate Development). C. after Cerfontaine, others after Sobotta. A. The ovarian egg showing cortical plasm. The first polar body is being pinched off, and the spindle for the second maturation division is formed. B. The cortical layer forming the perivitelline membrane on the surface of the egg within the vitelline membrane. C. The fusion of the vitelline membrane and perivitelline membrane to form the fertilization membrane is complete, but the latter has not yet left the surface of the egg. D. The extruded and fertilized egg. The fertilization membrane is beginning to leave the surface of the egg.

c. Cortical layer. e. Endoplasm. m. Fused vitelline and perivitelline membranes, *i.e.* the fertilization membrane. p. Perivitelline space. s. Spermatozoön. v. Vitelline membrane. I. First polar body. II. Second polar spindle.

lecithal. Near the close of the growth period a thin vitelline membrane is formed.

MATURATION AND FERTILIZATION

The First Maturation Division. — When the egg has reached full size the first maturation division takes place at the animal pole. It is preceded in this instance by the formation of tetrads (see page 45). Immediately following this division, preparations for the second one begin, and proceed as far as the metaphase (Fig. 54, *A*). The process pauses in this stage until after fertilization. Meanwhile as the first polar body separates from the egg it pushes through the vitelline membrane, carrying a small portion of the latter with it.
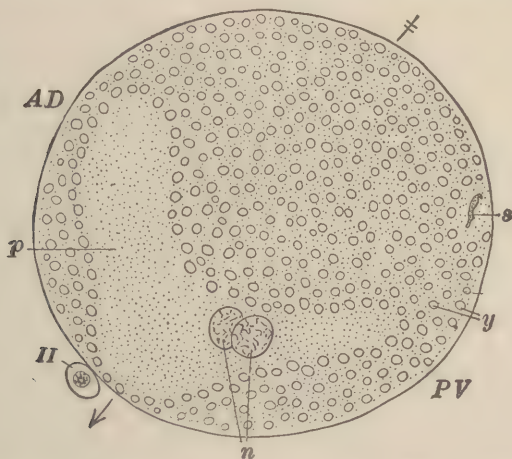
Fig. 55. — Diagram of a median sagittal section through the fertilized egg of Amphioxus at the stage of the fusion of the egg and sperm pronuclei. From Kellicott (Chordate Development). After Cerfontaine. The arrow marks the direction of the chief or polar axis.
AD. Antero-dorsal region (in terms of future embryo). *n.* Egg and sperm pronuclei. *p.* Region relatively free from deutoplasm. *PV.* Postero-ventral region. *s.* Remains of tail of spermatozoön. *y.* Yolk or deutoplasmic bodies. II. Second polar body.

Hence it is entirely free and is often lost (Fig. 54, *D*). At the same time the egg bursts out into a portion of the gonocœl next to the atrium.

Spawning and Fertilization. — Spawning occurs throughout the spring and summer, and always toward evening, while the animals are swimming. At this time muscular contractions occur in the walls of the above gonocœl cavities and thus cause the eggs to burst through

these walls, at certain points termed the *cicatrices*. The cutis wall of the atrium is also ruptured in these regions and the eggs thus reach the atrial cavity and from thence the exterior. As soon as the egg comes in contact with the sea water a second membrane is formed inside the first. It is called the *perivitelline membrane,* and is separated from the original covering by a slight space.[1] The new membrane seems to be formed from the vacuolated cytoplasm (cortical plasm) at the surface of the ovum, with which for a short time it remains in close contact. It is at first of a fluid consistency, but after a brief exposure to the action of the water it begins to toughen. This process starts in the region of the animal pole, from whence it soon spreads rapidly around the egg.

Meanwhile the latter has become surrounded by sperm which have been shed into the water near the female. One or more of these sperm now penetrate the outer or vitelline membrane, cross the intervening space, pierce the inner membrane, and enter the egg. Such entrance is generally effected near the vegetal pole where the perivitelline covering remains longest in a fluid condition. As soon as the sperm have reached the egg itself, however, the toughening of this membrane is rapidly completed. Also it seems to fill the space between the egg and the original vitelline membrane with which it apparently becomes fused (Fig. 54, *B, C*). The fused membranes thus form together what may be termed a *fertilization membrane,* and this presently becomes separated from the surface of the egg by the usual ("true") perivitelline space (Fig. 54, *D*).

The Second Maturation Division: Fusion of the Egg and Sperm Nuclei. — The entrance of the sperm is a stimulus which causes the second maturation division to become completed, and the second polar body is cut off. Meanwhile the sperm head (i.e., the sperm nucleus) enlarges so that it is equal in size to the egg nucleus. The two nuclei then meet and fuse in the usual manner. Any other sperm which may have gained entrance degenerate without further activity and the process of fertilization may be said to be complete.

[1] This space is literally perivitelline, and is often referred to as such. It differs from the space more usually so named, however, in that it exists previous to fertilization, and also in that it is, at this time, separated from the egg by a separate covering, the perivitelline membrane.

EGG SYMMETRY AND SEGMENTATION

Symmetry and Orientation. — The polarity of the egg; i.e., the establishment of the animal and vegetal poles, is traceable to its point of attachment in the ovary; i.e., the vegetal pole is on the unattached side. The entrance of the sperm then establishes a third point and so determines a median plane. The egg now becomes oriented with the animal pole about 30° below a horizontal plane passed through the center of the ovum. This position is important to
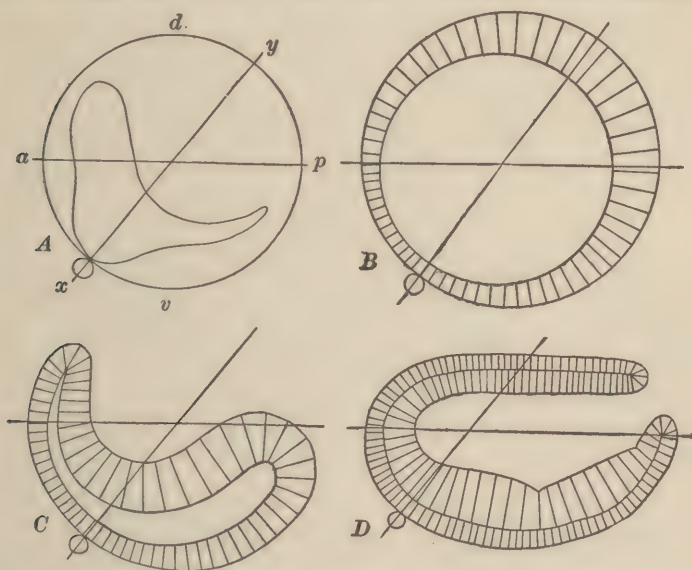
Fig. 56. — Diagrams illustrating the relations between the adult axes and the axes of the egg and early stages. From Kellicott (Chordate Development). After Cerfontaine. A. Fertilized egg. B. Fully formed blastula. C. Gastrulation begun. D. Fully formed gastrula. Note posterior elongation.

a–p. Anterior posterior axis of adult. d, v. Dorsal and ventral surfaces of adult. x–y. Chief egg axis (x animal pole, v vegetal pole).

bear in mind because it is apparently retained throughout subsequent development. It thus happens that the anterior end of the animal comes to lie, not at the animal pole of the egg, but at a point about 30° above it in the median plane. The opposite or posterior end on a like basis lies not at the vegetal pole, but 30° below it and also, of course, in the median plane. To put it in another way, speaking in terms of the anterior and posterior of the future animal, the animal

pole of the egg is antero-ventral and the vegetal pole postero-dorsal (Fig. 56). We are now prepared to describe the process of segmentation, keeping always in mind the tilted position of the egg's polar axis.

Segmentation. — Segmentation in Amphioxus is of the total or holoblastic type, but is not quite equal. The first division occurs about an hour after fertilization, and the second about an hour after
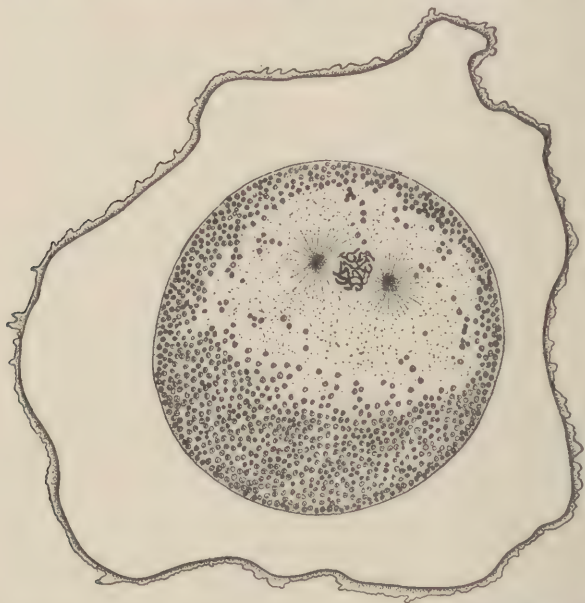
Fig. 57. — Prophase of first cleavage figure in Amphioxus. From Kellicott (Chordate Development). After Sobotta. Inner and outer membranes fused and separated from the egg by a wide space called the perivitelline space.

the first. Subsequent divisions follow each other at intervals of fifteen or twenty minutes.

First Cleavage. — The first cleavage spindle becomes situated within the cone of clear protoplasm, where its position is such that its center is cut at right angles by the median plane of the egg. The line of cleavage, therefore, coincides with that plane, and divides the ovum into equal right and left halves (Fig. 57).

Second Cleavage. — The second cleavage is at right angles to the first, and is also approximately meridional. It is not exactly so, however, since its plane lies a little above both the animal and vegetal

poles. There are thus produced two antero-dorsal cells slightly smaller than the postero-ventral pair (Fig. 58, *A*).

Third Cleavage. — Beginning with this division, the cleavage of the various cells is no longer exactly synchronous, the smaller cells tending to divide earlier than the larger. It is partly this fact which
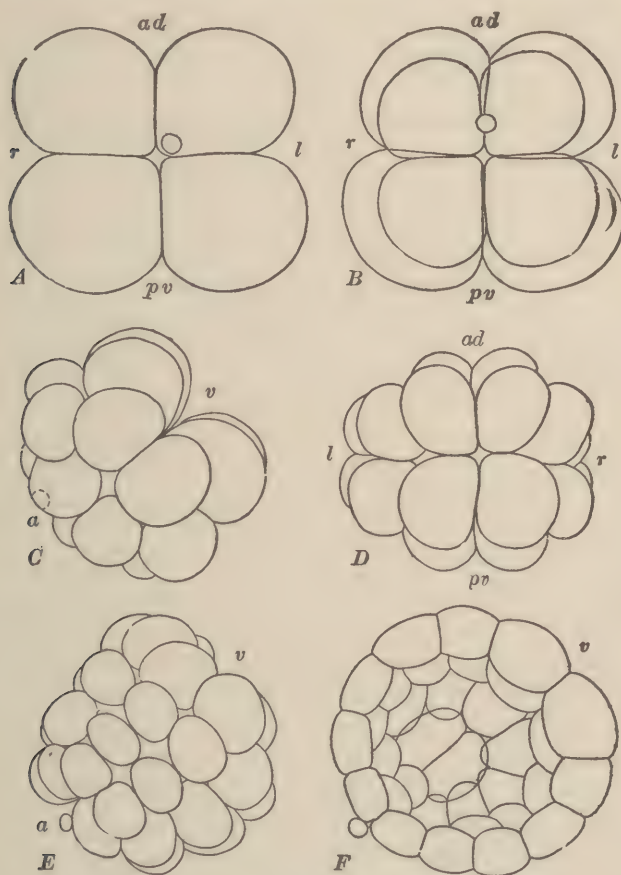
Fig. 58. — Cleavage in Amphioxus. From Kellicott (Chordate Development). After Cerfontaine. A. four-cell stage viewed from animal pole. The two antero-dorsal cells are the smaller. B. Eight-cell stage viewed from animal pole showing the four sizes of the cells. C. Sixteen cells viewed from the left side. D. Thirty-two cells viewed from vegetal pole. E. Thirty-two passing into sixty-four cells, viewed from the left side. F. Optical section of right half of young blastula. About **128** cells.

a. Animal pole. *ad*. Antero-dorsal. *l*. Left. *pv*. Postero-ventral. *r*. right. *v*. Vegetal pole.

presently causes the regularity of cleavage to disappear. Nevertheless, for the next division or two the interval between cleavages is long enough so that the process is entirely completed in the larger cells before it is started again in the smaller ones. Thus it is usually possible to speak of an actual eight, a sixteen, and a thirty-two-cell stage.

The third cleavage plane is at right angles to the first two; i.e., it is latitudinal with respect to the animal and vegetal poles of the egg. It is not quite equatorial, however, since it is situated slightly nearer the animal pole. The result is the production of four pairs of cells, the two at the animal pole being termed *micromeres,* and the two at the vegetal pole *macromeres* (Fig. 58, *B*). As regards the orientation of these cells relative to the future embryo, the upper pair of mic.o-meres are anterior, and the lower pair ventral, while the upper pair of macromeres are dorsal and the lower pair posterior. From the above account also, it is evident that the anterior pair of micromeres and the dorsal pair of macromeres are respectively slightly smaller than the other pair of the same type.

Fourth Cleavage. — The plane of this cleavage in each cell is at right angles to that of the third; that is, the cleavage is again longitu-dinal relative to the poles of the egg. It is not, however, meridional either in the micromeres or macromeres. Instead the new division plane of the micromeres is nearly perpendicular to the median plane of the original ovum, while that of the macromeres is almost parallel to this plane. The result as regards the micromeres is two rows of four cells each, lying parallel with the median plane, each row being arched with the convex side toward the animal pole. The macromeres on the other hand also form two rows of four cells each, but perpendicular to the median plane. Likewise though these rows are arched toward the animal pole, the direction of the arch, like that of the rows, is at right angles to the rows of micromeres (Fig. 58, *C*). A clear idea of the situation as regards the arching may be gained as follows: Let the left hand represent the micromeres and the right hand the macromeres, the rows of cells in each case lying parallel to the length of the fingers. If now each hand is cupped and the palm of the left placed over the back of the right, with the fingers of the two hands at right angles to one another, the relation of micromeres to macromeres will be indicated.

Fifth Cleavage. — This division is typically again latitudinal, so

that there result two layers of micromeres and two of macromeres. Thus, in all, there are thirty-two cells arranged in eight meridional rows with respect to the original animal and vegetal poles, the micromeres toward the former and the macromeres toward the latter. It should be added, however, that this cleavage is very likely to be irregular (Fig. 58, *D. E*).

The Blastula. — Subsequent cleavages are quite variable, but always result ultimately in the formation of a *blastula* containing a central space, the *blastocœl* or *segmentation cavity*. This stage may be considered as having been attained when the number of cells has reached about 128. By this time these cells are no longer round, but have become packed against one another so as to form a single epithelial layer. Toward the animal pole; i.e., antero-ventrally, this wall of the blastocœl is formed, of course, of smaller cells, and hence is somewhat thinner than the postero-dorsal wall. Between the two regions there is a gradual gradation in thickness. As a result of this, the blastocœl appears to be slightly eccentric in the antero-ventral direction (Fig. 58, *F*).

GASTRULATION, FORMATION OF CENTRAL NERVOUS SYSTEM, MESODERM, NOTOCHORD, AND CŒLOM

GASTRULATION

The exact nature of the process of gastrulation in Amphioxus has been the subject of much dispute. This is owing partly at least to the minute size of the larva at this time, and the consequent difficulty of determining just what occurs. As heretofore, the account which will be chiefly followed is that of Cerfontaine, according to whom the main processes involved are invagination, involution, and epiboly, together with the resulting phenomenon of concrescence.

Invagination. — The first event to be noted is a flattening of the vegetal side of the blastula, which is followed by a swinging inward of this whole area. That is, to a certain extent it is, as it were, pushed in as in the typical illustration of this process. It is not, however, a simple act of invagination, but rather, as stated, a swinging in. The inward movement is greatest at the *dorsal lip* of the future blastopore and increasingly less at the *lateral lips,* while the *ventral lip* serves

as the hinge to which the swinging plate of cells is attached (Fig. 59 *A, B, C, D*).

Involution. — It is clear that if this type of invagination were all that occurred a break must necessarily result between the vegetal plate and the dorsal and lateral lips. That such a break does not result is apparently due to the simultaneous occurrence of involution.[2] This, in turn, is supposed to be made possible as usual by means of extremely active cell division just at the margin of the dorsal lip, decreasing in its extent toward either side.[3] The new cells thus produced are then constantly moved over the blastoporal margin in the manner characteristic of this process, and from there they extend to the edge of the in-swinging vegetal plate (Fig. 59, *D*).

Epiboly. — Following, and to some extent accompanying, the foregoing processes, there also occurs a gradual lengthening of the gastrula in the direction of its anterior posterior axis. This is presumably made possible by cell divisions which are occurring everywhere in both ectoderm and endoderm, but more particularly perhaps in the blastoporal lips (*germ ring*). Likewise, coinciding with these movements, the blastopore itself is becoming smaller. From this description it may now be recognized that the activities of the layer of ectoderm and endoderm and the lip of the blastopore are not in themselves fundamentally different from those which characterize ordinary epiboly in eggs possessing relatively abundant yolk. Hence for this reason the above process is sometimes so considered. This case differs from one of epiboly as strictly defined, however, in one important respect just suggested; i.e., there exists no yolk mass which the advancing layers may cover. It is, so to speak, overgrowth with nothing but a cavity to overgrow (Fig. 59, *E, F, G*).

As a result of the above processes the *gastrula* soon loses its spherical shape, and becomes a short double-walled tube, the outer wall being *ectoderm* and the inner wall *endoderm*. The blastocœl has disappeared and the cavity which remains is the *archenteron*, opening posteriorly by a small *blastopore*. The dorsal region of this tube is somewhat flattened, but during the lengthening the ventral surface has remained markedly convex. From this fact it also follows that the blastoporal opening is quite near to the dorsal side (Fig. 59, *G, H*).

[2] MacBride claims that no real involution occurs in Amphioxus; that the cells of both ectoderm and endoderm simply multiply.

[3] Morgan and Hazen state that cell division is, at this time, equally active everywhere, rather than especially in the blastoporal lip.
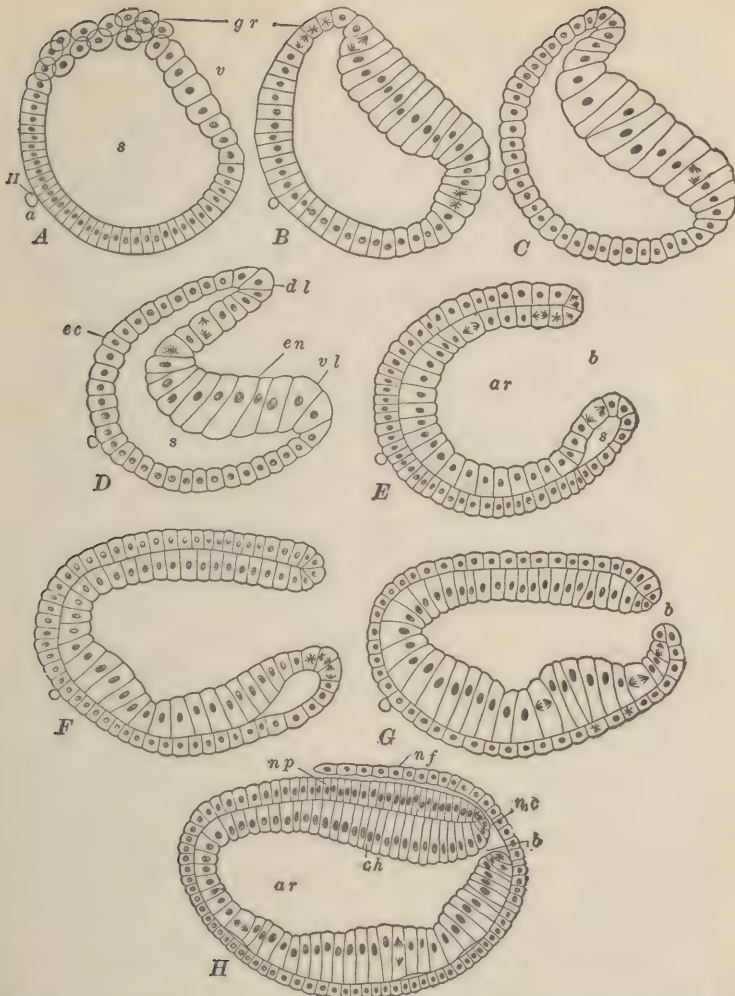
Fig. 59. — Gastrulation in Amphioxus. From Kellicott (Chordate Development). After Cerfontaine. A. Blastula showing flattening of the vegetal pole and the rapid proliferation of cells in the postero-dorsal region (germ ring). B. Flattening more pronounced; mitoses in cells of the germ ring. C. Commencement of the infolding (invagination) of the cells of the vegetal pole. D. Continued infolding and inflection, or involution, of ectoderm cells in the dorsal lip of the blastopore. The blastocœl becoming obliterated and the archenteron being established. E. Invagination complete. Continued involution in the dorsal lip of blastopore. Mitoses in germ ring. F. Constriction of blastopore and commencement of elongation of the gastrula. Remnants of blastocœl in ventral lip of blastopore. G. Gastrulation completed. Continued elongation, and narrowing of blastopore. H. Neurenteric canal established by overgrowth of neural folds. Continued mitosis in germ ring.

a. Animal pole. ar. Archenteron. b. Blastoporal opening. ch. Rudiment of notochord. dl. Dorsal lip of blastopore. ec. Ectoderm. en. Endoderm. gr. Germ ring. nc. Neurenteric canal. nf. Neural fold. np. Neural plate. s. Blastocœl or segmentation cavity. v. Vegetal pole. vl. Ventral lip of blastopore. II. Second polar body.

Concrescence. — Lastly it must be noted that in connection with the process of modified epiboly, there also occurs a quite typical instance of concrescence. Thus, as the blastopore decreases in circumference the material of its lips is paid into the mid-dorsal line in a perfectly characteristic manner. Hence, as is so frequently the case, it appears that the substance along this line is to a considerable extent derived from the blastoporal lip or germ ring.

During the above processes the ectoderm cells develop cilia which vibrate and thus cause the larva to rotate slowly within the egg membrane.

THE CENTRAL NERVOUS SYSTEM

The Neural Plate and the Neural Folds. — The beginnings of the central nervous system become evident at this time in the following manner. Along the middle of the flattened dorsal side of the elongated gastrula, a relatively wide strip of ectodermal cells becomes slightly depressed, thus marking it off from the ectoderm on either side. It is called the *neural* or *medullary plate*. At the same time the ectoderm along each side of this plate becomes slightly elevated, and these elevations then begin to grow toward one another above the plate. As this process continues the ectoderm constituting the elevations becomes separated from that at the margins of the plate. and the former gradually approach each other until they meet and fuse along the median line (Fig. 60, *A, B, C*). Thus the medullary plate itself is entirely roofed over, and during the process it is customary to speak of the free edges of the two approaching layers of ectoderm as the *medullary* or *neural folds*. As a matter of fact, however, these layers obviously (Fig. 60) involve none of the actual medullary plate, and only constitute the outer half of a true fold. Hence the neural folds, as here indicated, are but partly homologous with the similarly named structures in most higher forms (see below). It should now be added that the phenomena just described do not occur everywhere simultaneously. The depression of the neural plate begins just in front of the blastopore, and extends anteriorly, while the fusion of the neural folds begins slightly further forward and extends both ways.

The Neuropore. — Inasmuch as the neural folds pass around the lateral margins of the blastopore to meet each other on its ventral side, the coming together of their edges completely roofs over this

opening. The archenteron, however, still communicates with the
exterior. This is accomplished by means of the space extending along
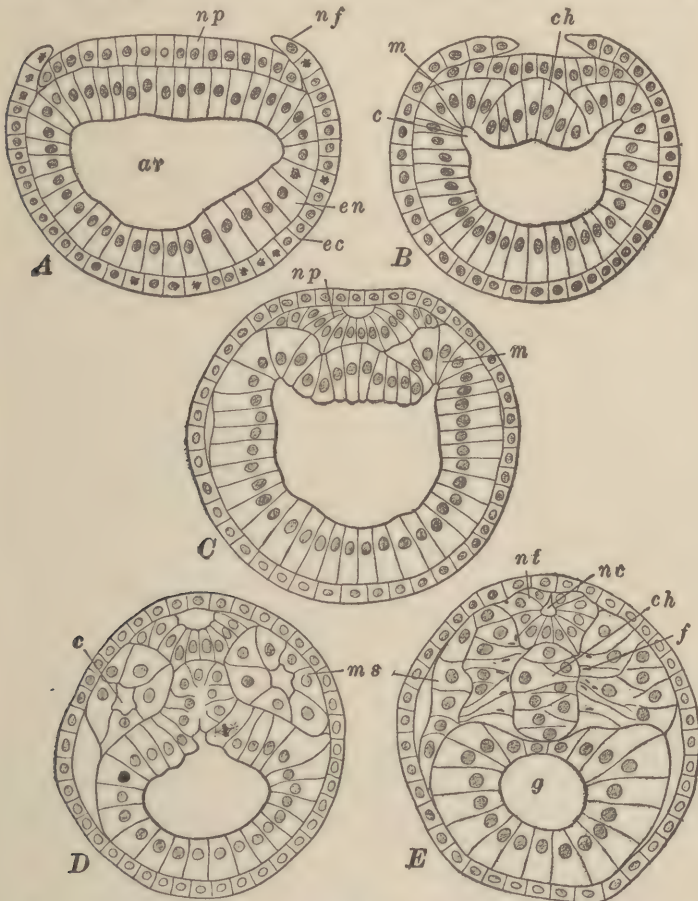
Fig. 60. — Transverse sections through young embryos of Amphioxus, showing
formation of nerve cord, notochord and mesoderm. From Kellicott (Chordate Develop-
ment). After Cerfontaine. A. Commencement of the growth of the superficial ecto-
derm (neural folds) above the neural or medullary plate. B. Continued growth of
the ectoderm over the neural plate. Differentiation of the notochord, and first indica-
tions of the mesoderm and enterocœlic cavities. C. Section through middle of larva
with two somites. Neural plate folding into a tube. D. Section through first pair of
mesodermal somites, now completely constricted off. E. Section through middle of
larva with nine pairs of somites. Neural plate folded into a tube. Notochord com-
pletely separated. In the inner cells of the somites, muscle fibrillae are forming.
   c. Enterocœl. ch. Notochord. ec. Ectoderm. en. Endoderm. f. Muscle fibrillae.
g. Gut cavity. m. Unsegmented mesoderm fold. ms. Mesodermal somite. nc. Neuro-
cœl. nf. Neural fold. np. Neural plate. nt. Neural tube.

the back of the embryo between the neural folds above and the medullary plate beneath. This space leads from the blastopore forward to the point where the folds are still in the process of uniting, and here opens to the outside. This opening is termed the *neuropore,* and is
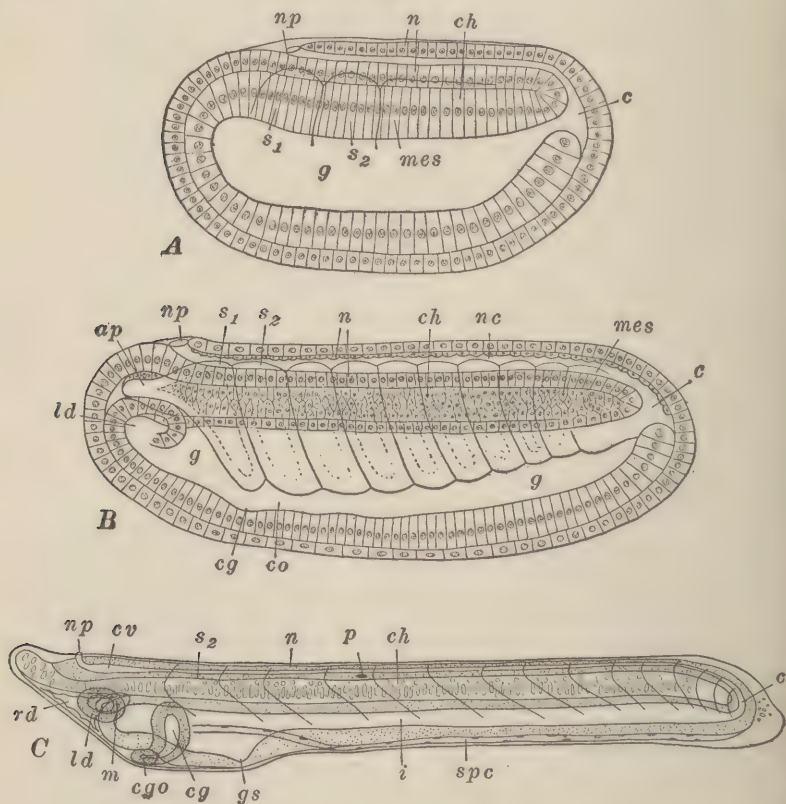
Fig. 61. — Optical sections of young embryos of Amphioxus. From Kellicott (Chordate Development). After Hatschek. The cilia are omitted. A. Two-somite stage, approximately at the time of hatching, showing relation of neuropore and neurenteric canal. B. Nine-somite stage, showing origin of anterior gut diverticula. C. Fifteen-somite stage. End of the embryonic period.

ap. Anterior process of first somite.   *c.* Neurenteric canal.   *ch.* Notochord (or its rudiment, in A).   *cg.* Club-shaped gland (or its rudiment in B).   *cgo.* External opening of club-shaped gland.   *co.* Cœlomic cavity of somite.   *cv.* Cerebral vesicle.   *g.* Gut cavity (enteron, mesenteron).   *gs.* Rudiment of first gill slit.   *i.* Intestine.   *ld.* Left anterior gut diverticulum (preoral pit in C).   *m.* Mouth.   *mes.* Unsegmented mesoderm.   *n.* Nerve cord (or its rudiment, in A).   *nc.* Neurocœl.   *np.* Neuropore.   *p.* Pigment spot in nerve cord.   *rd.* Right anterior gut diverticulum (preoral head cavity in C).   *s₁ s₂.* First and second mesodermal somites.   *spc.* Splanchnocœl (body cavity).

constantly advancing as the meeting of the folds continues. At the time of hatching, which occurs eight to fifteen hours after fertilization, this point is generally somewhat anterior to the middle of the embryo (Fig. 61).

The Neurocœl and the Neurenteric Canal. — When in approximately this condition as regards the nervous system, the young embryo breaks out of the egg membranes. Further development of this system then proceeds as follows. The process of roofing over the medullary plate is completed so that the neuropore is carried almost to the anterior end of the animal. The center of the neural plate is then somewhat further depressed, while its edges [4] are bent upward and inward until they meet (Fig. 60, *C, D, E*). There is thus formed within the old space between the former flat plate and the fused neural folds, a new tube — the *neural tube*, containing a canal, the *neural canal* or *neurocœl* (Fig. 61, *B*). The inner surface of this canal is evidently that of the original neural plate, and hence as might be expected, is lined with cilia. From the method of its formation also, it is clear that anteriorly the neurocœl will open to the exterior at the neuropore and that posteriorly it will still communicate with the archenteron through the blastopore. This posterior passageway through the blastopore into the neurocœl is now termed the *neurenteric canal*. Both neurenteric canal and neuropore remain open throughout the embryonic period; i.e., until the mouth is formed.

Later, the anterior portion of the neural tube widens somewhat to form the rudiment of a brain, while within the tube at this and other points, pigment spots appear. These are probably light receptors.

This is as far as it is necessary to consider the development of the nervous system in Amphioxus. In comparing this development with that of most higher Chordates there will be found a fundamental similarity. There is one variation in detail, however, which, though it has already been indicated, deserves a further word of emphasis. In all those cases where the neural tube is formed by so-called neural folds (see the footnote on this process in the Teleosts, Chapter II), it is only in Amphioxus that the completion of the real tube occurs later than, and hence separately from, the overgrowth and fusion of the folds. Indeed, as will appear from reference to Fig. 52, in all true Vertebrates in which the tube arises by fold formation, the edge of the

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[4] These edges are really homologous with the inner or nervous portion of the neural folds as described in the Frog (see below).

plate remains united to the edge of the outer layer of overgrowing ectoderm until the folds from opposite sides meet. Thus in these latter cases the structures so named are truly folds, instead of being only the outer half of a fold as in Amphioxus.[5]

The Development of the Mesoderm, the Notochord, and the Cœlom

Shortly after the nervous system has begun to develop and some time previous to hatching, the mesoderm starts to form. As might be expected from what has already been said about the primitiveness of Amphioxus, this is a case in which the enterocœlic method partially prevails. Even here, however, it will appear that the process occurs in a typical manner only in the anterior part of the animal. It may be described as follows:

The Origin of Enterocœls and Somites. — The mid-dorsal region of the archenteron is occupied from end to end by a strip approximately six to nine cells in width. On each side of this median strip there is another strip or band from three to five cells wide, and it is with these latter bands that we are now concerned. They also extend the entire length of the archenteric cavity, and at its posterior end they pass around the lateral lips of the blastopore to meet on its ventral side. There is nothing at first to mark out the bands from the remainder of the endoderm. Presently, however, each band begins to become folded along almost its entire length, anterior to the blastopore. This longitudinal fold occurs in such a way that the groove thus formed faces the archenteron, while the convex side is next to the ectoderm (Fig. 60, *B*, *C*). The grooves are deeper anteriorly and in this region there develops in each a transverse partition. Thus on each side a small pocket, cut off from the remaining posterior portion of the groove, is formed, and these two pockets are the first *enterocœlic pouches*. It is evident that they have been formed, as described in our general account of this method, by an outpushing from the archenteron. In this case, however, the growth of the transverse partition was required to actually complete them. The cavities of these pouches or pockets remain for a time in communication with the

[5] The peculiar method by which the neural tube is formed in Amphioxus must probably be regarded as specialized rather than primitive. Upon this same basis some authorities do not homologize the overgrowing ectoderm with any part of a true neural fold.

archenteron. Later, however, each pouch is entirely cut off from
the endoderm. It then exists as a more or less cubical block of cells
between the wall of the archenteron and the outer ectoderm, and is
known as a *mesodermal somite*. Within the center of the somite is
the enterocœlic cavity which was originally part of the archenteron
(Fig. 60, *D*). This cavity represents the beginning of the *cœlom*. It
should be clearly noted that the term somite as used in connection
with Amphioxus applies both to the myotomal region (segmental
plate in true Vertebrates), and to the lateral plate, instead of only
to the former. This will become more apparent presently.

In the meantime more transverse partitions are forming in each
groove and thus giving rise to more pairs of somites. The second
pair is similar to the first and is formed just before the larva hatches.
At this time, however, neither pair has yet become entirely separated
from the archenteron. After the hatching the process continues,
though with certain differences in detail with respect to the more pos-
terior somites. Thus in those immediately back of the first two
pairs, the cavity of the pouch; i.e., the enterocœl, closes just after the
somite is cut off from the endoderm, but later opens again. In those
further back the closure occurs even before the cutting off takes place.
Finally just anterior to the fourteenth or fifteenth pair, the folds of
the mesoderm fuse with one another as they arise, so that no real
groove ever exists. Thus the somites in this region originate from solid
bands of cells. Back of this region still more somites are later de-
veloped, also from solid mesodermal bands, until eventually as many
as sixty-one pairs are formed. The more posterior mesoderm from
which these later somites arise, however, is not at any time a part
of the archenteron. It originates in a somewhat different manner, as
will appear in the succeeding paragraph. Nevertheless, though many
somites are thus solid in the beginning, all ultimately develop cœlomic
spaces. In connection with these spaces, they give rise to other
parts, whose development will be taken up after consideration of the
two types of mesoderm and of the notochord.

The Gastral and the Peristomial Mesoderm. — It has been stated
that the cells which compose the dorso-lateral bands of the archen-
teron are indistinguishable at first from the cells which form the re-
mainder of the endoderm. In view of their fate, however, it is evidently
possible to regard the former cells as really different from those of
the true endoderm from the very time of their formation; that is, it

is possible to regard them as potential mesoderm before they be-
come actually such by the formation of the somites. Hence in this
instance the term *mesentoderm* may be aptly applied to those portions
of the archenteric wall which constitute these bands. To go another
step, it will further be recalled that these bands not only form the
dorso-lateral sides of the archenteric cavity, but are also continued
in the form of undifferentiated material around the lips of the blasto-
pore. On this basis, therefore, it is possible for a time to divide the
potential mesoderm into two kinds; i.e., that which forms the dorso-
lateral bands, and that which forms the extensions of these bands
about the blastopore. The mesoderm which is derived from the former
is then termed *gastral mesoderm,* and that which is derived from the
latter *peristomial mesoderm.* Upon this basis it later becomes evi-
dent that only the mesoderm which comprises the first two pairs of
somites is strictly gastral. Posterior to these pairs and anterior
to the 14th or 15th, on the other hand, the mesoderm arises from
material which has originated in the lips of the blastopore, and is
hence, according to definition, peristomial. It should be noted, how-
ever, that before this peristomial material really gives rise to meso-
derm, it first becomes mesentoderm; that is, by means of the process
of concrescence it is brought into the same relative position in the
dorso-lateral walls of the archenteron as the originally mesentodermic
material immediately anterior to it. In other words this peristomial
substance really becomes gastral before it becomes changed into
somites, so that the latter are only, as it were, secondarily peristomial
in their origin. It was indicated in the preceding paragraph, how-
ever, that the mesoderm posterior to the 14th and 15th somites is
derived from material which has never actually formed a part of the
archenteric wall; i.e., has never been gastral in position. Instead, this
mesoderm is derived directly from material originating in the lips
of the blastopore, and paid immediately into the mesodermal bands
from which the somites in this region are forming as the embryo elon-
gates. Hence such mesoderm may be said to be rather more strictly
peristomial than that derived from material which was first peristomial
and then gastral.

The Notochord. — In connection with the origin of the mesoderm
it will be recalled that between the two lateral bands from which it
arises there lies a median strip of cells. During the elongation of the
embryo this strip is constantly being added to by cells inflected at the

dorsal blastoporal lip. It is from this strip that the notochord develops in the following manner.

At the time of hatching it is still a virtually flat band. Presently, however, the sides of the band begin to bend downward, while its median region arches upward (Fig. 60, *B*). This continues until the sides have met each other, thus forming a solid laterally compressed rod two cell layers in width (Fig. 60, *D*). This rod is the rudimentary *notochord*. The cells from the two layers soon grow across the mid-line and become interlocked with one another, while at the same time the rod becomes entirely cut off from the archenteron. The edges of the endoderm which formerly bordered the outer margin of each lateral mesodermal band then grow together and thus completely roof in the primitive gut (Fig. 60, *E*). Finally vacuoles appear in the cells of the rod which is presently transformed into the typical notochordal tissue.

The processes thus described begin between the first and second somites and extend both anteriorly and posteriorly. Posteriorly the notochord ceases at the neurenteric canal, while anteriorly it reaches to the extreme end of the embryo in front of the brain (Fig. 61, *C*). In this last respect Amphioxus differs from other Chordates in which the notochord always stops beneath the mid-brain.

The Further Development of Somites and Cœlom

By the time five or six pairs of somites have been formed, it becomes evident that only the members of the first pair and the upper parts of the second are exactly opposite one another. Posterior to this the somites of the left side are more and more in advance of their mates on the right, until soon they alternate. This is a feature peculiarly characteristic of Amphioxus. It is also at about this point, or a little later, that the first two pairs become entirely separated from the endoderm, and at approximately the same time there appears another characteristic peculiar to this animal. This involves the forward extension of a portion of each somite of the first pair at the level of the notochord. It is said that the walls of this pair of extensions later go through the same processes as are described below for the walls of the somites.

The Lateral Plate. — At the stage of fourteen or fifteen somites certain further changes begin to appear in the more anterior pairs.

In each somite the entercœl becomes larger, while the walls of the ventral portion below the level of the notochord become thinner. At the same time this portion begins to lengthen in a postero-ventral direction, the region thus affected being known as the *lateral plate*.

The outer wall of this plate next to the ectoderm is called the *somatic* or *parietal* mesoderm, while the inner wall next to the enteron is *splanchnic* or *visceral* mesoderm. The part of the enterocœl which lies between them is the *splanchnocœl* or true cœlom. The lateral plates on each side of the embryo continue to grow ventrally until they finally meet. Presently the ventro-median wall which at first separates the splanchnocœls of the two sides largely disappears, as well as the walls separating the successive splanchnocœls of the same
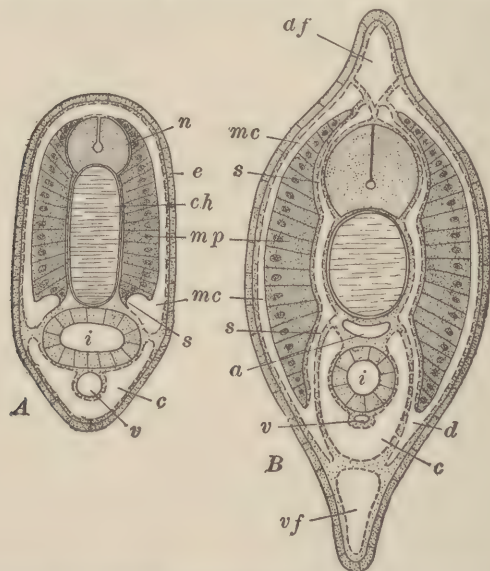
Fig. 62. — Diagrams of transverse sections through Amphioxus larvæ. From Kelli-cott (Chordate Development). A. Through the body region of a larva with five gill slits, showing separation of myocœl and splanchnocœl (cœlom). B. Through the region between atriopore and anus of young individual, shortly after metamorphosis, showing relations of sclerotome. After Hatschek.

a. Dorsal aorta. c. Cœlom (splanchnocœl). ch. Notochord. d. Dermatome. df. Dorsal fin cavity. e. Epidermis. i. Intestine. mc. Myocœl. mp. Muscle plate (myotome). n. Nerve cord. s. Sclerotome. v. Subintestinal vein. vf. Ventral fin cavity.

side. Thus the splanchnocœl or cœlom becomes continuous throughout the entire lateral and ventral region of the animal.

The Myotomal Region. — While this is going on in the lower portion of each somite, the upper portion on a level with the notochord is assuming the < shape characteristic of the adult. It is also becoming thicker, largely as a result of the horizontal flattening of its cells in the wall adjacent to the notochord. These cells presently become differentiated as muscle cells, extend throughout the length of the somite, and nearly obliterate the enterocœl in this upper region. The thickened muscular tissue of each somite is then called a *myotome*, while the slight enterocœlic space still remaining between the latter and the outer unthickened wall is termed a *myocœl* (Fig. 62). Later, between the myotome and the lateral plate there develops a horizontal partition which acts as a boundary between the two regions. Eventually also there grows out from the ventral region of the myotomal portion of each somite a fold of tissue which presently becomes divided into two parts. One part then extends upward between the myotome and the notochord and nerve cord as the *sclerotome*. The inner layer of this sclerotomal part finally forms the *skeletogenous sheath* for the latter structures, while its outer layer forms the covering or *fasciæ* for the inner sides of the myotomes themselves; the latter have no fasciæ on their outer sides, as they do in the Craniates. The other portion of the original fold meanwhile extends outward and downward between the somatic layer of the lateral plate and the ectoderm. This fold, together with the outer unthickened wall of the upper or myotomal region, is known as the *dermatome*. Its upper myotomal portion gives rise to the *cutis layer* of the integument in the dorsal part of the animal, while the fused inner and outer sheets of the fold constitute the same layer ventrally. These points should be kept in mind, in connection with the development of homologous parts in the higher Vertebrates.

The Anterior Gut Diverticula. — Although it is not strictly connected with the formation of the somites, we may mention in closing the appearance of certain diverticula of the archenteron, which in their early stages are not unlike enterocœls.[6] When about seven pairs of somites have been formed, there arise a pair of narrow outpushings from the anterior end of the archenteric cavity (Fig. 63, *A*). These later become pinched off from that cavity and each develops in its own peculiar fashion. The right one becomes greatly enlarged, as-

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[6] By some authorities (Hatschek, MacBride) these structures are regarded as actual, though modified, mesodermal somites.

sumes a median position, and occupies the whole of the space beneath the chorda and in front of the enteron. The left remains smaller and finally acquires an opening to the outside of the head known as the *pre-oral pit* (Fig. 61, *C*).

The later development of Amphioxus is too highly specialized to help us much in an understanding of the higher and more typical Chordates. It will therefore be omitted. Those students who are interested in the further history of this animal, however, will find a good brief account with references to original papers in Kellicott's *Chordate Development*. They should also note the references at the conclusion of this chapter.
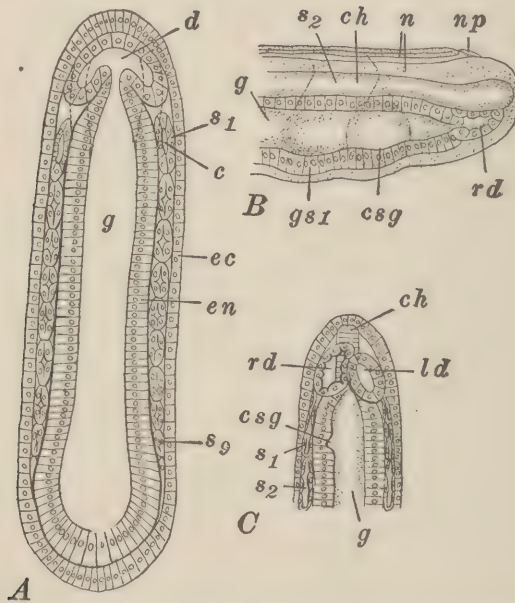
Fig. 63. — Sections through young Amphioxus embryos showing the origin of the anterior gut diverticula. From Kellicott (Chordate Development). After Hatschek. The cilia are omitted. A. Frontal section through embryo with nine pairs of somites. (See Fig. 61, B.) The dotted line marks the course of the gut wall ventral to the level of the section. B. Optical sagittal section through anterior end of embryo with thirteen pairs of somites, showing position of right anterior gut diverticulum. C. Same in ventral view.

c. Cœlomic cavity of somite. *ch*. Notochord. *csg*. Rudiment of club-shaped gland. *d*. Rudiment of anterior gut diverticula. *ec*. Ectoderm. *en*. Endoderm. *g*. Gut cavity (enteron, mesenteron). *gs1*. Rudiment of first gill slit. *ld*. Left anterior gut diverticulum. *n*. Nerve cord. *np*. Neuropore. *rd*. Right anterior gut diverticulum. s_1 s_2 s_9, First, second and ninth mesodermal somites.

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PART II

THE DEVELOPMENT OF THE FROG

THE FROG: FROM THE PRODUCTION OF THE GERM CELLS THROUGH GASTRULATION

The embryology of the Frog, *Rana sp.*, will be taken up as the first example of the development of a true Vertebrate, being a valuable object for such study for the following reasons: In the first place its earlier history furnishes an excellent transition between the corresponding stages in Amphioxus and those in animals which are more highly evolved. Secondly, the later development of the Frog is also very suggestive from an evolutionary point of view. Thus it illustrates in a striking manner the transformation of a purely aquatic gill-breathing Vertebrate into one which breathes largely by lungs, and is capable of extended existence on land. Thirdly, in the course of its development the Frog shows the origin of practically all of the fundamental Vertebrate systems. Yet in many cases these systems remain in a rather primitive condition, and are thus helpful to an understanding of the complications which are met with in other types. Fourthly, the development of the Frog is of importance from the general zoölogical viewpoint because of the significant work which has been done upon it in the past. Lastly, there are also certain practical considerations. The living material is usually available at an appropriate time of year, it is easy to handle, and the young can be readily cared for under laboratory conditions.

THE REPRODUCTIVE ORGANS OF THE ADULT, OÖGENESIS, AND THE EXTRUSION OF THE OVA

THE MALE ORGANS

The Testes. — There are two testes in the Frog, each one lying in the dorsal region of the cœlom, close to the kidney (Fig. 64). Each is enveloped by the peritoneal epithelium, which is fused above the organ into a two-layered sheet of tissue, like a mesentery. This

sheet attaches the testis to the body wall and is termed the *mesorchium*. In appearance each testis is a white ovoid body which may be a half inch or so in length. In some species in which the sperm are produced continuously, the size of the organ remains fairly constant. In others, however, in which spermatogenesis is chiefly confined to the breeding seasons, the dimensions vary considerably. This variation is nevertheless relatively small compared to what always occurs in the ovary.

In structure each testis consists essentially of a mass of *seminiferous tubules*. These are grouped into lobules and the latter again into lobes, separated by thin partitions of supporting or connective tissue. This tissue also covers the whole organ in a coat called the *tunica albuginea,* outside of which is finally the peritoneum. The walls of the tubules are lined internally with follicle or nutrient cells (*Sertoli cells*), while between the latter and the lumen of each tubule come groups of germ cells in various stages of development, those in any given group being in approximately the same stage. As the cells of a group reach the condition of spermatids their heads are gathered together and the tips embedded in a Sertoli cell. Finally when fully ripe the spermatozoa are liberated into the tubular lumen.

Fig. 64. — The male urinogenital system of the adult Frog (*Rana pipiens*) viewed from the ventral side. The testes in this case are medium sized. The urinary bladder and rectum have been dissected out and reflected posteriorly. Otherwise in the ventral view they would cover the lower part of the reproductive organs. Note the large fat bodies as compared with those in the female. Also note the rudimentary oviducts. In many species of Frogs these ducts do not develop so far in the male as in *R. pipiens*. They have no known function in this sex.

ad. Adrenals. *bv.* Blood vessel. *cl.* Cloaca. *fb.* Fat bodies. *k.* Kidney (mesonephros). *od'.* R u d i m e n t a r y oviduct. *r.* Rectum. *sv.* Seminal vesicle. *t.* Testis. *ub.* Urinary bladder. *ur.* Ureter, in the male serving also as a vas deferens. *ut'.* Rudimentary uterus. *ve.* Vasa efferentia.

To the anterior end of each testis is attached a *fat body,* composed of a mass of yellow streamers. Its function is uncertain. Inasmuch as the animals do not eat during

the breeding season, however, it may serve as an extra supply of nutrient material to be drawn on at this time.

The Sperm Ducts. — The tubules of each testis open into about a dozen fine ducts, the *vasa efferentia*. These lead through the substance of the kidney and empty into its duct which thus acts as both *ureter* and sperm duct (*vas deferens*). The two vasa deferentia are dilated just before entering the cloaca to form the *seminal vesicles*. In these, the sperm are stored previous to discharge.

THE FEMALE ORGANS

The Ovaries. — The ovaries are also paired organs and occupy the same relative position as the testes (Fig. 65). As in the case of the latter, each is suspended from the body wall by a double sheet of peritoneal tissue in this instance called the *mesovarium*. Unlike the testes, however, the ovaries always vary greatly in size and appearance, depending upon the time of year. During the breeding season they are filled with ripe ova and occupy a large share of the body cavity. They are lobulated in form, and exhibit a characteristic black and white speckling, due to the color of the eggs. At

Fig. 65. The female urinogenital system of the adult Frog (*Rana pipiens*) viewed from the ventral side. The left ovary has been removed, showing the fat body kidney and oviduct upon that side. The right ovary full of nearly mature eggs remains in place. Note that the fat body is smaller than in the male, having presumably suffered depletion during the development of the eggs. The urinary bladder and rectum are omitted from the figure, but occur in the same position as in the male.

inf. Infundibulum. *o.* Ovary. *od.* Oviduct. *ut.* Uterus. Other abbreviations as in figure 64.

other times they are comparatively small and inconspicuous.

In structure, the ovary consists of a number of compartments, whose outer walls are formed of connective tissue or *stroma*. Within

each compartment are the ova in various stages of development, depending upon the season. The youngest germ cells, or *oögonia*, are in the process of dividing, while the older cells or oöcytes, which predominate near breeding time, are undergoing growth and maturation. Each of these oöcytes is surrounded by a single layer of flattened cells which constitute its *follicle*. Outside of this is another layer termed the *theca*, which serves to attach the ovum to the wall of its compartment. Between the two layers are blood vessels.

Attached to the anterior end of each ovary is a fat body similar in appearance, and presumably in general function, to those connected with the testes.

The Oviducts. — These are long convoluted tubes whose size and convolutions are somewhat increased during the breeding season. They open anteriorly into the cœlom by a ciliated funnel, the *infundibulum*. Posteriorly they open into the cloaca. Throughout the greater part of their length the walls are quite thick, especially during breeding time. This thickening is due to the hyper-development of numerous simple tubular glands which secrete the gelatinous covering of the eggs. The lumen of the ducts is lined by ciliated epithelium. At the posterior end, each duct widens and its walls become thinner and very elastic. These dilated regions are known as the *uteri*, and they serve for storing the ova just prior to extrusion. Each duct is covered by a layer of peritoneum and slung from the dorsal body wall in the same manner as are the gonads.

OÖGENESIS

The Oögonia. — The breeding season referred to above occurs in the spring or early summer. At this time the ovaries are emptied of ripe eggs, and the relatively few oögonia which remain begin to multiply to produce the eggs for the next season. These occur in nests, and in each such nest only one cell is destined finally to become an ovum, the others constituting its follicle. As soon as an ovum has become definitely differentiated as such, and its follicle formed, the period of growth and membrane formation sets in.

The Growth Period. — When this period has been reached the young ovum or oöcyte, as it may now be called, begins to accumulate yolk. This first appears in the form of granules which gather about the attraction sphere on one side of the nucleus (Fig. 66, *D*). Under
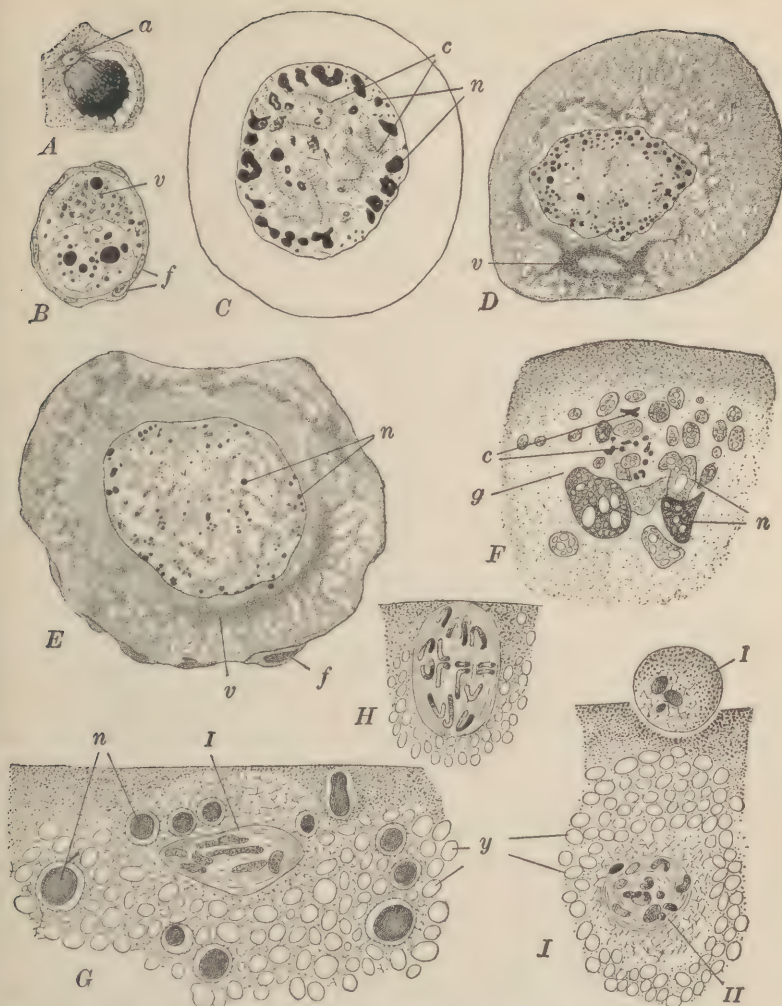
Fig. 66. — Oögenesis in the Frog (*R. temporaria*). From Kellicott (Chordate Development) A–E, after Lams. F–I, after Lebrun. A. Primary oöcyte in synizesis. B. Primary oöcyte with vitelline substance (yolk) of mitochondrial (chromidial?) origin in the cytoplasm. C. Primary oöcyte showing feathery chromosomes and chromatin nucleoli. D. Primary oöcyte with ring-like vitelline mass. E. Primary oöcyte showing cytoplasm in two zones. F. Nuclear region of primary oöcyte after dissolution of the nuclear membrane showing the small chromosomes and large chromatin nucleoi. Egg still in ovary. G. First polar spindle in primary position. From egg in body cavity. H. First polar spindle in metaphase. From egg in uterus. I. First polar body formed and second polar spindle forming. From eggs in uterus.

a. Attraction sphere. *c.* Chromosomes. *f.* Follicle cells. *g.* Contents of germinal vesicle. *n.* Chromatin nucleoli. *v.* Vitelline substance of mitochondrial (chromidial) origin. *y.* Yolk plates. I. First polar spindle (polar body in I). II. Second polar spindle.

such circumstances this sphere is often termed the " yolk nucleus,"
though obviously it has nothing in common with a genuine nucleus.
Presently this sphere breaks down and the yolk granules about it
are scattered through the cytoplasm (Fig. 66, *E*). Soon, however,
they pass to one side of the egg, while the true nucleus moves to
the other. Thus the ovum becomes distinctly telolecithal. The
polarity so produced is then further emphasized by the accumulation
of pigment granules within the nuclear half. These granules which
are apparently a product of metabolism occur in the superficial proto-
plasm, and extend to the equator or a little below it. Thus the
nuclear or animal pole of the egg is nearly black, while the vegetal
pole is a creamy white. This difference accounts for the speckled
appearance of the ovary, already noted. The ovum has meantime
been acquiring two membranes. The inner membrane is an extremely
delicate and close-fitting envelope secreted by the egg itself. It is
therefore a true *vitelline* membrane, but is so thin that its actual
existence is denied by some investigators. The outer covering is thin,
but tough, and is formed by the follicle. Hence it is a secondary
membrane or *chorion*.

While the ovum has been growing and acquiring its membranes
the nucleus has been passing through the stages preliminary to the
first maturation division. In the female Frog these stages vary some-
what from what has been described as typical. The chief difference
consists in the fact that after synizesis (Fig. 66, *A*), the chromatin
threads seem to disappear. Therefore when the heterochromosomes
for the first maturation division later develop, instead of being de-
rived from these threads, they apparently arise from certain chromatin
nucleoli (Fig. 66, *D, E, F*).[1]

Before these chromosomes actually form, however, certain other
events occur, as follows: The nucleus moves quite close to the ani-
mal pole, and the latter becomes slightly flattened. Also, in many
cases the pigment covering this pole withdraws to a small extent just
above the nucleus, giving rise at this point to a somewhat lighter
area termed the *fovea*. The egg has now reached a diameter of from
1.5 to 3 mm., depending upon the species of Frog, and is ready for
ovulation.

[1] According to some authorities the threads do not really lose their identity,
but merely become very fine and difficult to see. They later give rise to the
chromatin which forms the chromosomes.

The series of processes leading to this result have taken place during the summer, and are virtually completed before the time of hibernating arrives. The eggs remain in this condition until the period of spawning in the following spring.

OVULATION TO FERTILIZATION

Ovulation. — When spring arrives the frogs enter the water; and there the female is clasped firmly by the forelimbs of the male. This embrace, known as *amplexus*, lasts throughout the spawning period, so that the male is always in a position to fertilize the eggs whenever they are extruded. It is thought likely also that the stimulus afforded by this contact helps to bring about ovulation.

To accomplish the latter process, the ovarian follicle breaks, and the ripe ovum is forced out through the epithelial covering of the ovary into the cœlom. No matter in what region of the body cavity this act may occur, ciliary action on the peritoneum serves to convey the egg to the mouth or infundibulum of the oviduct. This is also ciliated and the ovum is drawn into the duct.

The First Maturation Division. — Before following the progress of the egg further it will be necessary to return for a moment to processes occurring within it.

At about the time of ovulation the nuclear membrane dissolves, and shortly afterward the chromosomes of the first maturation figure arise from the nucleoli, as indicated above. As this figure forms, another peculiarity of maturation in the female Frog becomes evident, for neither centrosomes nor asters are visible. Out of the fibrillar protoplasm, however, a spindle develops, division of the chromosomes occurs, and the first polar body is pinched off while the egg is in the upper part of the oviduct. This body lies just beneath the chorionic membrane. Immediately following this the spindle for the second division develops, and the division proceeds to the metaphase. In this stage it remains until after fertilization.

The Tertiary Egg Coverings. — As the egg passes down the oviduct from the infundibulum to the uterus the walls of the duct secrete about it three or four layers of albuminous material which constitute a tertiary covering. These layers are hardly distinct as such at this time, but as will appear below they become so after contact with the water.

Spawning. — Within about two hours after entering the infundibulum the egg reaches the uterus where it may remain for a day or two until this portion of the duct is full. The accumulated mass of ova are then expelled into the water, and in the common American Wood Frog a single such act of expulsion usually completes the process of spawning. In some varieties of Frog, however, the expulsive act is followed by another accumulation of eggs, and the spawning period is thus prolonged. Hence, though in American Frogs its duration is usually not more than a few days, in some European species it may continue for over a week, the process in any case being retarded by cold. As already noted the male remains in amplexus throughout this time, although in those instances where repeated expulsions are the rule, the actual extrusion of eggs generally occurs only in the early mornings of successive days. The total number of eggs spawned in a season varies in different species of Frogs and in different individuals. Thus in *Rana temporaria* it runs from 1000 to 2000, while in *Rana esculenta* it may be anywhere from 5000 to 10,000.
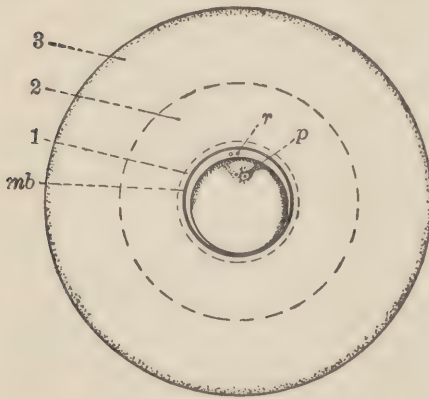
Fig. 67. — Egg of Frog a short time after laying and fertilization, showing the swollen egg membranes. From Ziegler (Lehrbuch, etc.), after O. Schultze.

mb. The chorion, presumably plus the vitelline membrane. *p.* Pigmented penetration path of the spermatozoön. *r.* Polar bodies lying in perivitelline space. **1, 2, 3,** inner, middle and outer albumenous membranes or layers of " jelly."

The Effect of Water on the Tertiary Membrane. — After spawning the membrane indicated above of course comes in contact with the water, and by absorbing it, begins immediately to swell. This action progresses rather rapidly at first, so that within two or three minutes the jelly-like covering has increased from $1/6$ the diameter of the egg to about $1/2$ that diameter. In fifteen minutes it generally equals the egg diameter; thereafter the swelling becomes slower. At this point, if fertilization has not occurred the absorption of water by the jelly is said almost to cease. If fertilization has taken place,

however, the swelling process may continue for several hours until the thickness of the jelly is as much as twice the width of the ovum.

This thickening reveals more clearly the three or four layers of which the jelly membrane is really composed. The innermost is a thin dense stratum applied closely to the chorion, and sometimes erroneously referred to as the chorion itself. Next comes a rather thick and watery layer, and finally one which is both thick and firm. When a fourth is present it is thin and fibrous; it does not occur outside, but just beneath the thick firm layer which is always outermost.

Although some species of Frogs have elaborate habits connected with the care of the eggs, the common Frog does not. When fertilized, the eggs are simply deposited and left to their fate. On this account the thick envelope of jelly which they possess appears to exercise several important functions. In the first place it serves to attach them to each other and to debris, so that they are not readily washed about. It protects them from mechanical injury, and also appears to be distasteful to water snails and perhaps other animals. Finally it functions as a lens to concentrate the sun's rays and at the same time prevents the outward radiation of heat, thus raising the temperature of the egg.

FERTILIZATION AND EGG SYMMETRY

FERTILIZATION

The Penetration of the Sperm. — As the eggs are extruded by the female, the male Frog immediately discharges over them the seminal fluid. This fluid contains thousands of spermatozoa, and hence the eggs tend to be surrounded by them. Many of these pierce the outer jelly, but usually one of them is slightly in advance of its fellows and thus arrives first at the surface of the egg itself. As soon as it has started to enter some change is effected in the egg so that the remaining sperm are unable to pass beyond the jelly. Polyspermy is thus abnormal in the Frog and when it occurs the course of development is interfered with.

The entrance of the sperm always occurs in the animal hemisphere of the egg, and usually, according to some authorities, about 40° from the pole. Aside from these limitations, however, there is apparently nothing which fixes the point of penetration; that is, this point may be located on any one of the infinite number of meridians which may be imagined to pass from one pole of the egg to the other.

The Perivitelline Space. — The penetration of the ovum by the sperm seems to cause the egg to give up a certain amount of its fluid. In any case, whatever its source, fluid does collect at this time between the chorion and the surface of the ovum. It is indeed presumably inside the vitelline membrane if the latter exists, and hence the space containing this fluid is as usual termed the *perivitelline* space. Its formation releases the egg from the grip of its coverings so that it is free to rotate within them. Under these conditions if the lighter animal pole is not already uppermost it presently becomes so. Since this pole is covered with black pigment, its new position is probably of advantage in more efficiently converting the concentrated sun's rays into heat.

The Entrance Path. — In the case of the Frog the whole spermatozoan enters the ovum, and it usually requires a minute or two for it to get entirely inside. The tail then disintegrates, and the head and middle piece travel steadily along a path which is generally approximately a radius of the egg, leaving a trail of pigment behind them (Fig. 68, *B*). This is the *penetration* or *entrance path,* and as the head and middle piece move along it, the usual rotation of these parts occurs, thus placing the later structure in the lead. At the same time the head is enlarging to form a typical nucleus.

The Second Maturation Divison. — Meanwhile the stimulus of the entrance of the sperm has incited the completion of the second maturation division of the egg nucleus which had paused in the metaphase. After throwing off the second polar body, the egg nucleus withdraws from the surface of the ovum, usually to a position in the egg axis. The sperm nucleus then proceeds toward it.

The Copulation Path and the Fusion of the Egg and Sperm Nuclei. — As suggested under the general topic of fertilization, the course followed by the sperm immediately after its penetration of the egg (i.e., the entrance path) may not be directed exactly toward the egg nucleus. In those instances where it is not, therefore, the point where the sperm does start to move directly toward this nucleus is marked by a slight change in its course. The second portion of the sperm path which thus arises, as has already been noted, is then called the *copulation path,* and like the first portion, in the case of the Frog, it is marked by a trail of pigment (Fig. 68, *A*).

Proceeding along this second path the sperm nucleus presently meets that of the ovum. Meanwhile the middle piece has initiated the

formation of a centrosome and aster, and before the meeting of the pronuclei occurs this has divided into two. The division has taken place at right angles to the copulation path, and hence as the nuclei come together the axis joining the centrosomes coincides with their plane of union.
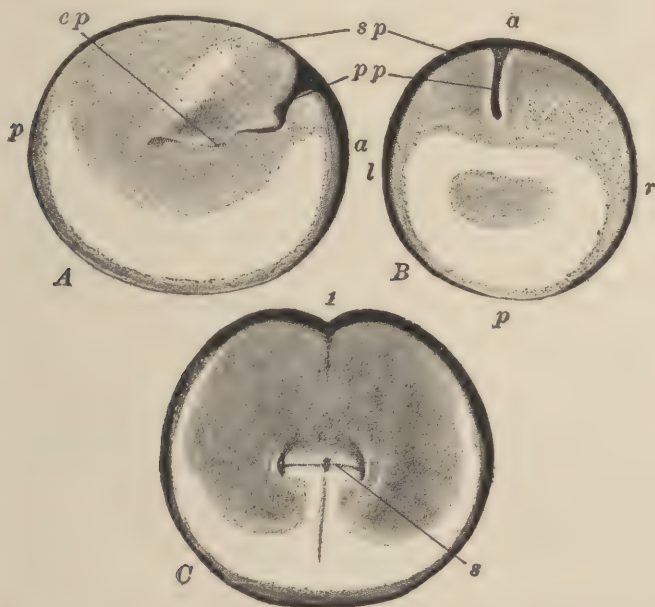
Fig. 68. — Sections through the egg of *R. fusca*, showing the penetration and copulation paths, and the symmetry of the first cleavage plane. From Kellicott (Chordate Development). After O. Schultze. A. Sagittal section through the egg before the appearance of the first cleavage. B. Frontal section of the same stage as A, showing the symmetrical distribution of the egg materials. C. Frontal section through egg in two-cell stage, showing the symmetry of the egg; the penetration path is not shown.
a. Anterior. *cp*. Copulation path. *l*. Left. *p*. Posterior. *pp*. Penetration path. *r*. Right. *s*. Remains of the first cleavage spindle. *sp*. Superficial pigment. 1. First cleavage furrow.

The Symmetry of the Ovum and Its Significance

The causes which determine the symmetry of any ovum and the relation which this symmetry bears to cleavage and to the symmetry of the embryo are subjects of fundamental importance for the understanding of development. They have therefore received considerable attention in different groups of animals, and among Vertebrates the Frog's

egg has seemed particularly well adapted for such study. Hence it appears desirable in the case of this animal to make some mention of the results to which this study has led. It must be noted, however, that in spite of the work which has been done, there still exists some disagreement as to the exact facts, at least as regards certain details. In the interest of clearness, therefore, it seems best merely to state the main features of this phase of development in the Frog according to one view, the accounts followed being chiefly those of Roux and Jenkinson. Certain points in the descriptions given by these authors, however, are regarded by many as of doubtful accuracy; these have been omitted from the body of the text and included in a footnote.

The First Plane of Symmetry. — Before the egg is fertilized it is radially symmetrical about an axis passing through its poles. The penetration of its surface by the sperm, however, confers upon it a bilateral symmetry. That is to say, the point of this penetration, together with the polar axis, determines a plane which, save for the possible eccentricity of the egg nucleus, divides the ovum into equal halves. It may be termed, therefore, the *sperm entrance point plane.* The existence of this plane of symmetry, determined solely by the egg axis and the sperm entrance point, however, is brief. Other factors presently enter which determine a second plane, according to some authorities closely correlated with the first (see below), and developed in the following manner:

The Second Plane of Symmetry. — As the sperm travels along the first part of its path within the egg, it seems to cause certain disturbances in the egg substance. The result is a more thorough separation between yolk and cytoplasm, and an apparent streaming of the latter in the direction of the sperm. This flow seems to cause a withdrawal of pigment granules from along the border of the pigmented animal hemisphere on the side of the egg from which the flow is taking place; i.e., the side approximately opposite to that upon which the sperm entered. The result is the appearance upon that portion of the pigmented border of a lighter strip termed the *gray crescent.* This crescent then continues to lose color until presently the region of its occurrence blends with the original white area. Thus the upward extent of the latter upon the side of the egg where the crescent was formed is proportionately increased (Fig. 69, *A, B*). The new plane of symmetry, therefore, is one which again passes through the egg axis and also bisects the gray crescent,
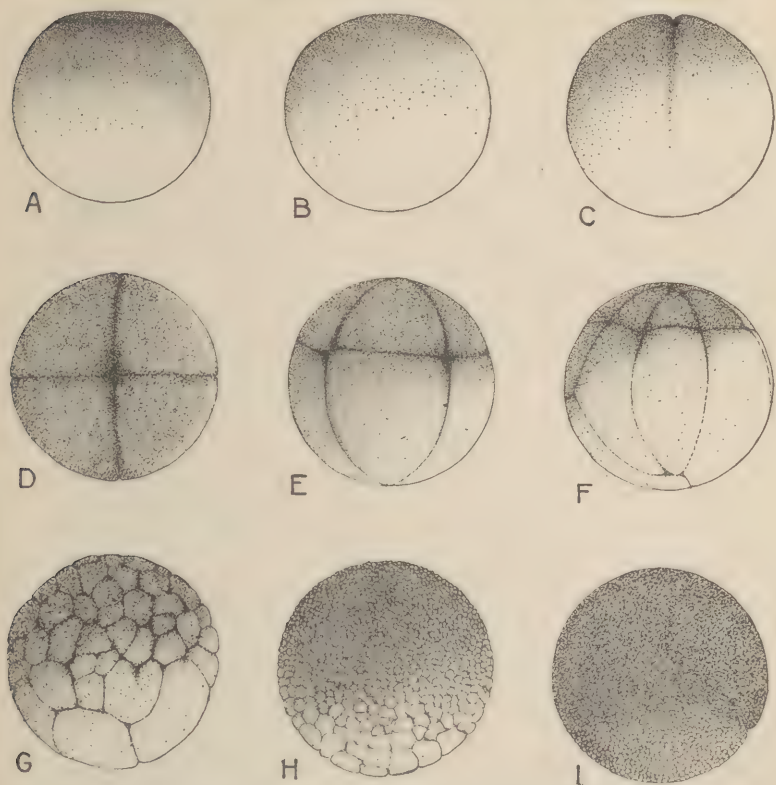
Fig. 69. — Cleavage stages and the beginning of gastrulation in the Frog's egg (*Rana pipiens*). The shading in this figure indicates the distribution of pigment, except along the lines of cleavage, where as usual it denotes shadow. A. The unfertilized egg. Note the even distribution of pigment in the animal hemisphere, there being as yet no gray crescent. B. Fertilization has taken place and the first cleavage is completed. The latter, however, is in the plane of the paper and therefore does not show. The egg is being viewed from its left side with respect to the orientation of the future embryo, and hence the region of the gray crescent (marking the posterior of the embryo) is at the right (posterior) of the figure. At this stage the region of the crescent has evidently become whitened and added to the original light area of the vegetal pole. C. An incomplete four cell stage, also viewed from the left side. The second furrow has not quite reached the vegetal pole. D. A view of C from the animal pole, with the region of the gray crescent (posterior) toward the right. E. An eight cell stage. The animal pole is again at the top of the page, and the vegetal pole at the bottom, but the future anterior region is turned slightly toward the observer, thus showing part of the first furrow. F. An approximate sixteen cell stage directly from the left side. The cleavage is obviously somewhat irregular. G. Between a 64 and 128 cell stage viewed from the left side. H. A virtually complete blastula from the left side. Note that the pigmented area is tending to move downward somewhat. I. An early gastrula from the left side. The cells in the animal hemisphere are too small and numerous to indicate separately. The beginning of the blastopore lip is visible as a slight notch in the lower left side of the figure.

or the increased area of white which replaces it. It may be called the *second* or *gray crescent plane,* and by virtue of its method of formation it will evidently have a decided tendency, as suggested above, to coincide with that of the sperm entrance point.[2]

The Relation of the Two Planes to Cleavage and to the Symmetry of the Embryo.

I. The Relation of the Sperm Entrance Point Plane to the First Cleavage Plane.—The direction of a cleavage plane in an egg is dependent, like the cleavage plane in any cell, upon the position occupied by the mitotic spindle; i.e., it will be at right angles to the longitudinal axis of the spindle. Now as already noted, the new centrosome and aster in the egg of the Frog form as usual just in front of the advancing sperm nucleus. They then divide into two, and thus give rise to a spindle, which, barring disturbing influences (see below), tends to lie at right angles to the copulation path as the sperm and egg nuclei meet in or near the egg axis. In the case of the Frog, moreover, the spindle is also subject to the action of gravity in such a way that its longitudinal axis is virtually always horizontal. It then follows that the first cleavage plane will be vertical, and that, in so far as the copulation path and entrance path lie in the same vertical plane with each other, it will pass through them and also through the sperm entrance point. In other words, under such conditions the sperm entrance point plane will coincide with that of the first cleavage, and as a matter of fact there appears to be a considerable tendency for this coincidence to occur. That it does not always do so, however, is probably due chiefly to three factors, as follows:

[2] Jenkinson asserts that there is really not a great deal of correlation between the actual entrance point plane and that of the gray crescent, but that there is considerable between the plane formed by the *entrance path* and that of the crescent. This is apparently due to the facts (1) that the entrance path of the sperm is not always along a radius of the egg, and (2) that the influence which forms the crescent is exerted by the sperm not only as it enters, but also as it travels along this path. Furthermore, according to this author, correlation between either of the planes connected with the sperm, and that of the gray crescent, is considerably lessened because of the fact that the position of the crescent is not entirely determined by the influence of the sperm. Instead its position is partly the result of a completely independent factor; i.e., the direction of light rays across the egg. Also it is thought by Jenkinson and other authors that pressure and gravity may exercise some influence upon the position of the second plane, and thus disturb its chances of coincidence with the first. Lastly it should be noted that Roux has described a slight rotation of the entire egg in the direction of the side of sperm entrance.

1. First, as already suggested, the entrance path may not be in the same vertical plane as the copulation path, and other things being equal it is the latter which fixes the position of the mitotic spindle.

2. The second disturbing factor is that of pressure upon the egg, a condition frequently present within a large egg cluster. Such pressure tends to change the shape of the egg, and at the same time to alter the position of its mitotic spindle. This follows in accordance with the generalization known as Hertwig's law, which states that the longitudinal axis of a mitotic spindle always tends to lie in the same direction as the longitudinal axis of the cell. In other words under such conditions the cleavage plane tends to be parallel to the direction in which pressure is being applied; i.e., perpendicular to the long axis of both spindle and cell, regardless of the direction of the copulation path.

3. A third factor which may occasionally prevent the coincidence of the sperm entrance point plane and that of the first cleavage is that of gravity. It has already been noted that this force seems always to operate in such a way as to cause the first division plane to be vertical, and this statement holds regardless of the position of the egg. Normally, however, this has no effect on the coincidence of the planes in question because the animal pole of the egg always tends to turn uppermost, thus allowing the vertical cleavage plane to pass through both the egg axis and the sperm entrance point. But in abnormal cases, where the egg axis is in some way prevented from becoming vertical, the cleavage plane may fail to pass through either the axis or the sperm entrance point.

II. The Relation of the Gray Crescent Plane to that of the First Cleavage. — In describing the origin of the gray crescent it was noted that it had a marked tendency to form on the side of the egg opposite to the sperm entrance point; i.e., in such a position that the first and second planes would coincide. But it has just been shown that, barring certain disturbing influences, there is also a considerable tendency for the first cleavage plane to coincide with that of the sperm entrance point. Hence it follows that the first cleavage plane is also likely to coincide with that of the gray crescent.

III. The Relation of the Gray Crescent Plane to the Symmetry of the Embryo. — It has been quite clearly shown that whatever may be the relation of the gray crescent plane to any other, it

always appears to coincide with the median longitudinal plane of the future embryo; i.e., the dorsal lip of the blastopore always develops along the border of the highest region of the gray crescent (see below). Whether this means that the latter plane is determined by the gray crescent, or whether it merely means that both planes are determined by other common factors is not certainly known.

IV. The Relation of the Sperm Entrance Point Plane to the Symmetry of the Embryo. — It has been said that in most cases the gray crescent plane coincides with that of the sperm entrance. Then, since the former always coincides with that of the median plane of the embryo, it follows that the median embryonic plane and the sperm entrance point plane will usually tend to coincide.

Summary. — It appears that the sperm entrance point plane and that of the gray crescent coincide in a considerable number of cases, while the latter always coincides with the median longitudinal plane of the future embryo. The plane of the first cleavage furrow usually coincides with all three of the above planes, but it may coincide with that of the sperm entrance point alone, with that of the gray crescent and embryonic symmetry alone, or with none of them. The reason for this lies in the fact that the position of the cleavage spindle is partly determined by forces which are independent of those determining the planes of the sperm entrance point and the gray crescent.

CLEAVAGE, GASTRULATION, AND THE FORMATION OF MESODERM, NOTOCHORD, AND MEDULLARY PLATE

It has already been suggested that in the Frog the character of the processes indicated is transitional; it serves to bridge the gap between the activities observed in the development of Amphioxus and those in some of the forms which are to follow. Not only is this true, but the character of the Frog's egg as regards its yolk content is also transitional. The egg of Amphioxus was telolecithal, but the amount of yolk was relatively slight. The egg of the Frog is telolecithal, but the amount of yolk is much greater. Finally, as will be seen, this condition is carried to its extreme in the Fish and Bird. As our study of these forms proceeds it will become increasingly apparent that this parallelism between the character of early development and the yolk content is not a coincidence. Rather,

as intimated in the first chapter, the latter very largely determines the former. The student then should keep this clearly in mind in attempting to understand the stages which follow as compared with corresponding stages in Amphioxus.

CLEAVAGE

The Early Stages. — In spite of the larger amount of yolk in the Frog's egg, segmentation is still holoblastic. Following the second cleavage, however, it is less nearly equal than in Amphioxus (Fig. 69). As has been stated the first division plane is vertical, and under normal conditions passes through the egg axis. This means that it divides the ovum into parts which are at least quantitatively similar. The particular meridian cut by the division is determined by factors noted above. The furrow which marks the beginning of this cleavage appears on the upper surface of the ovum about two and one half hours after fertilization and within an hour has extended around to the ventral pole. By the time it has reached this pole, the internal substance of the egg is also divided.

A period of " rest " ensues, and then, about three-quarters of an hour after the appearance of the first division, the furrows of the second become evident. This cleavage is also vertical and at right angles to the first. The furrow in each of the two hemispheres again begins approximately at the animal pole, often exactly so. When the latter is the case the upper ends of these furrows will evidently lie opposite each other and form a continuous line across the pole (Fig. 69, *D*).

Following the completion of the second cleavage, the third soon starts. It is horizontal, and in each of the four cells it lies about 60° below the animal pole. Hence its furrows form a virtually continuous line around the egg a little above the equator. This is the typical or at least the ideal condition (Fig. 69, *E*). There are, however, not infrequent variations.

The furrows of the fourth cleavage are in general vertical, and tend ideally to meet one another at the poles. This tendency, however, is seldom perfectly realized, even in the animal hemisphere. Thus in the latter half, the lines of division usually pass either to one side or the other of the polar center, while in the vegetal hemisphere this and other irregularities are even more marked. The ideal re-

sult, however, is sixteen cells, eight relatively small pigmented ones
above, and eight larger whitish ones below (Fig. 69, *F*).

The fifth cleavage, resulting in the formation of thirty-two cells,
is still more variable than the fourth. There is a tendency, how-
ever, for the furrows to be horizontal again, so as to form four
tiers of eight cells each. In the most regular instances the cells
of the two upper tiers are about equal to one another, and are of
course all pigmented. The cells of the third tier are about midway
in size between those above and those below them. They are ap-
proximately on the equator, and contain less pigment than the two
upper tiers. The lowest tier of all is formed of the largest cells,
which are practically without pigment.

The Blastula. — By the time the thirty-two-cell stage is reached
it is hardly possible longer to refer to this dividing sphere as an egg.
It may now, therefore, be termed the *blastula*. Within this *blastula*
is the *blastocœl* or *segmentation cavity*, which arises as follows:

From the first the cells into which the ovum has been divided are
pressed rather closely against one another so that their surfaces of
contact are flattened. This, it will be recalled, is contrary to the
rounded condition of the cleavage cells of Amphioxus. Even in the
Frog, however, the inner surfaces of the cells show some curvature.
As a result of this there is formed as early as the eight-cell stage, a
small space bounding the cells at their inner ends. This space is the
beginning of the blastocœl and by virtue of the unequal type of seg-
mentation it is necessarily located somewhat above the equator. This
relative position it continues to hold during most of its existence.
By the time the blastula stage is attained the blastocœl has increased
considerably in size, and this increase continues until about the
beginning of gastrulation. It becomes filled meanwhile with infilter-
ing water and with an albuminous fluid secreted by the surrounding
cells (Fig. 70, *A, B*).

Besides the increase in the segmentation cavity, other changes
also occur during the blastula period. Following the thirty-two-cell
stage, cleavage becomes entirely irregular, and sections of the blas-
tula show that cells are being split off internally. It soon becomes
evident too that division is going on more rapidly in the animal
than in the vegetal hemisphere. Sections of blastulas in this stage,
however, reveal the fact that some of the cells produced in the upper
part of the animal hemisphere tend to migrate toward the equator.

Here, moreover, cell division is particularly rapid, and hence it follows that while the roof of the blastocœl grows thinner, the equatorial region becomes thicker. Because of the mitotic activity in this latter region, as well as on account of its later history, it may be regarded as really the beginning of the *germ ring* (Fig. 70, *D*), and is presumably quite homologous with the structure of the same
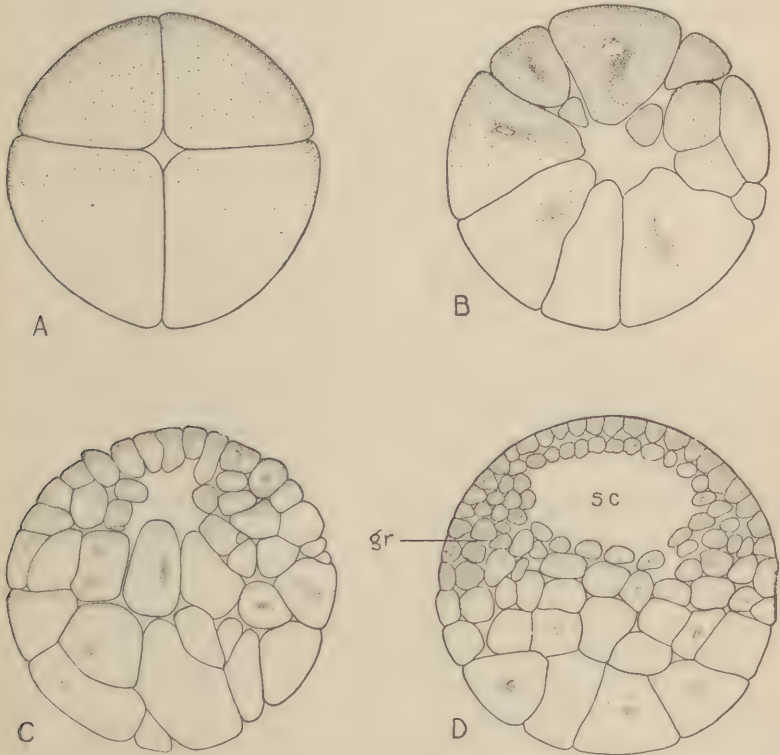
Fig. 70. — Median vertical sections of four cleavage stages in the Frog's egg. A. An eight cell stage. Note the small segmentation cavity or blastocœl. B. A later stage (about 32 cells) which may be called an early blastula. C. A later blastula. D. A still later blastula, showing a marked increase in the size of the segmentation cavity, and what may be termed the beginning of the germ ring.
gr. Germ ring. *sc.* Segmentation cavity.

name (i.e., *lip of the blastopore*) in Amphioxus. Here, however, the present relationship of this structure, as well as its future development, is considerably modified by the presence of so much yolk.

Finally, at what may be termed the end of the blastula period, the following conditions obtain: First the blastula is about one fifth larger than the original egg, the increase in size being mainly due no doubt to the absorption of water noted above. Secondly the superficial pigment has everywhere extended downward somewhat, thus decreasing the white area (Fig. 69, *H*). This extension having been approximately uniform, however, the latter region still reaches farther upward upon the side where it was originally augmented by the addition of the gray crescent. Thirdly, sections reveal the fact that on the side opposite to that which was marked by the gray crescent, the wall of the blastocœl is usually slightly thicker than it is elsewhere (Fig. 73, *A*). Lastly, it may be noted that a split has occurred in the roof of the segmentation cavity, so that this wall is composed of two sheets. The outer is the *epidermal layer;* the inner is called the *nervous layer*.

<center>GASTRULATION</center>

External Processes. — Upon the side of the blastula where the white area was increased by the addition of the region of the gray crescent, it has been noted that the pigment is still not quite so far down as upon the side opposite. Nevertheless, even at the former point this darkened area now extends markedly below the equator, and there presently appears along its border a short horizontal groove. The upper edge of this groove is the dorsal lip of the blastopore, and its advent indicates the beginning of gastrulation (Fig. 69, *I;* Fig. 71, *A*).

As this process continues it is accompanied externally by two phenomena. In the first place the groove gradually extends around either side of the gastrula, its margin always being rather sharply outlined by the limit of the pigmented area (Fig. 71, *A, B, C*). As suggested above, moreover, it is now evident that the upper edge of this groove; i.e., the blastopore lip, is everywhere identical with the lower margin of the germ ring. In the second place the groove and lip, accompanied by the ring, move nearer and nearer to the vegetal pole, the movement being much the greatest on the side where the lip first appeared; i.e., the future dorsal side, and becoming progressively less upon either hand. The first process; i.e., that of lateral extension, causes the groove to become curved so that it

has the shape of a crescent, and eventually the horns of this crescent meet each other so as to form a complete circle. A continuation of the second process; i.e., the down growth of the lip, and hence
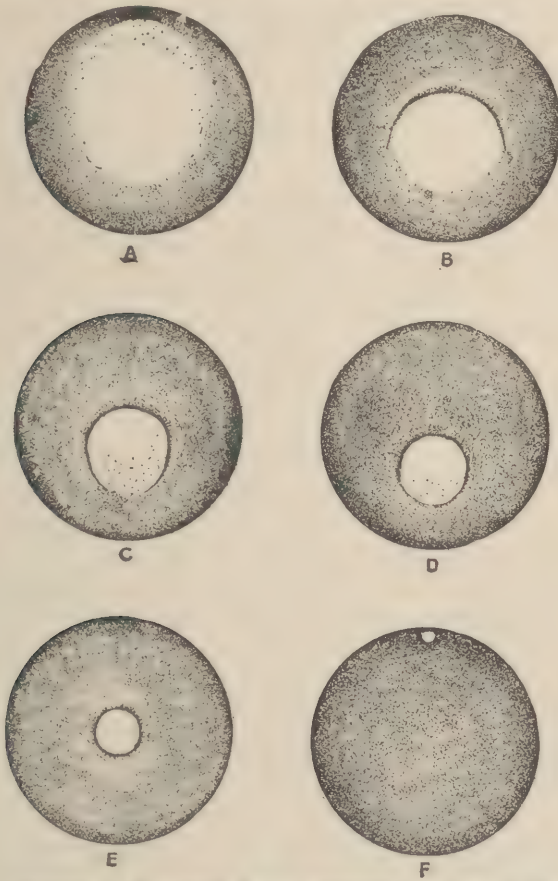
Fig. 71. — Diagrams of the closure of the blastopore in the egg of the common Frog (*R. temporaria*). From Jenkinson (Vertebrate Embryology). In A-E the egg is viewed from the vegetal pole, and in F, speaking in terms of the future embryo, from its ventral side. The dorsal lip is at the top of the figures. In D the ventral lip has just been formed and the blastopore is circular. In E the rotation of the whole egg has begun, and in F is complete.

also of the pigmented area, then results in a rapid diminution of the white region. Thus the latter is soon in the form of a circular spot which is being encroached upon from all directions (Fig. 71, **D, E, F**).

With the understanding that the germ ring is identical with the rim of the blastopore, the above events may now be interpreted as follows.

Epiboly and Concrescence. — The white region evidently occupies the position of the *blastopore.* The first appearance of the groove marks the beginning of overgrowth by the dorsal blastoporal lip, while the lateral extensions of this groove indicate the same process on the part of the lateral lips. Finally, as already noted, the ends of the grooves meet one another on the future postero-ventral side of the *gastrula,* and thus show that there also a slight downgrowth is taking place. The gradual advance of the dorsal, of the lateral, and finally of the ventral lips over the surface of the yolk is therefore a typical case of gastrulation by overgrowth or epiboly. It may be further stated that careful examination of the process in both normal and abnormal embryos indicates that here, as in Amphioxus, this overgrowth is accompanied by concrescence.[3] That is to say, the material in the lateral portions of the germ ring is constantly flowing together at the dorsal lip. Hence as the downgrowth of this lip progresses a broad band of the fused material derived from each lateral lip is left behind along the mid-dorsal line; from the method of its formation this band is narrower in front and wider posteriorly.

Rotation. — The above processes continue until the dorsal lip has passed over an arc somewhat greater than 90°, and the area of white; i.e., the blastopore, is reduced to a small circle. This area, therefore, will be situated rather beyond the original vegetal pole. At about this point, however, a curious change occurs. The entire gastrula begins to rotate about a horizontal axis lying at right angles to the original median plane of the egg. The direction of rotation is such that the dorsal lip is carried directly back over the path it has just travelled, so that when the rotation ceases it has passed slightly beyond the point from which it started (Fig. 72). Thus the blastopore, formed at approximately the vegetal pole, is now posterior, and the dorsal and the ventral lips are actually dorsal and ventral. From this it also follows that the original animal pole of the egg is to form the antero-ventral side of the future embryo, while

[3] As evidence of what occurs in normal embryos, it is interesting to note the condition of embryos which have been placed in certain concentrations of a solution of Na Cl. Here though concrescence of the germ ring fails to occur, differentiation in each half of it proceeds to a considerable extent.

the region formerly marked by the gray crescent is to form the dorsal side.

As regards the events so far described it is evident that gastrulation in the Frog is not essentially dissimilar to the same process in Amphioxus. The main differences are due to the presence of the large yolk cells. Thus, to cite one instance, if these were absent the germ ring would bound an opening just as in the former case. Here, however, this opening; i.e., the blastopore, is filled by these cells, which at this point are therefore termed the *yolk-plug*. As will presently appear, the phenomenon of rotation and various internal peculiarities are also due to the presence of so much inert nutrient material.
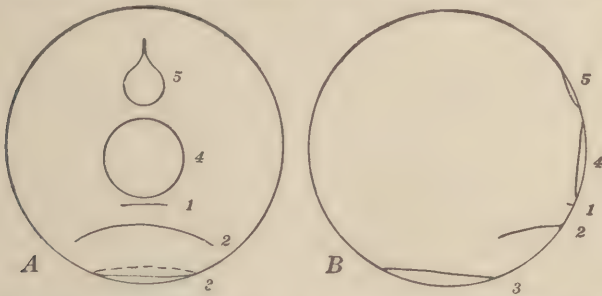
Fig. 72. — Diagrams of the Frog's gastrula showing the position of the blastopore at various ages. From Kellicott (Chordate Development). A. Posterior view. B. Lateral view. 1-5 indicate the successive positions and forms of the blastopore. The change in position is due both to the actual growth movements of the blastopore, and to the rotation of the entire gastrula.

Internal Processes. — While the above changes are apparent from the outside of the gastrula, sections through it at various stages will reveal important accompanying developments within. They are as follows:

Invagination. — As the external processes of gastrulation begin, meridional sections of the blastula (or early gastrula) bisecting the future dorsal blastoporal lip reveal the fact that the floor of the blastocœl nearest this lip is beginning to move upward slightly; i.e., since rotation has not yet occurred, toward the animal pole, leaving a very slight space between it and the outer wall (Figs. 73, *B;* 74, *B*). Later sections through other meridians, moreover, indicate that this process soon spreads toward either side in company with the external extension of the blastoporal lip, until presently the entire circumference of the floor is involved. Precisely to what this movement is due is not
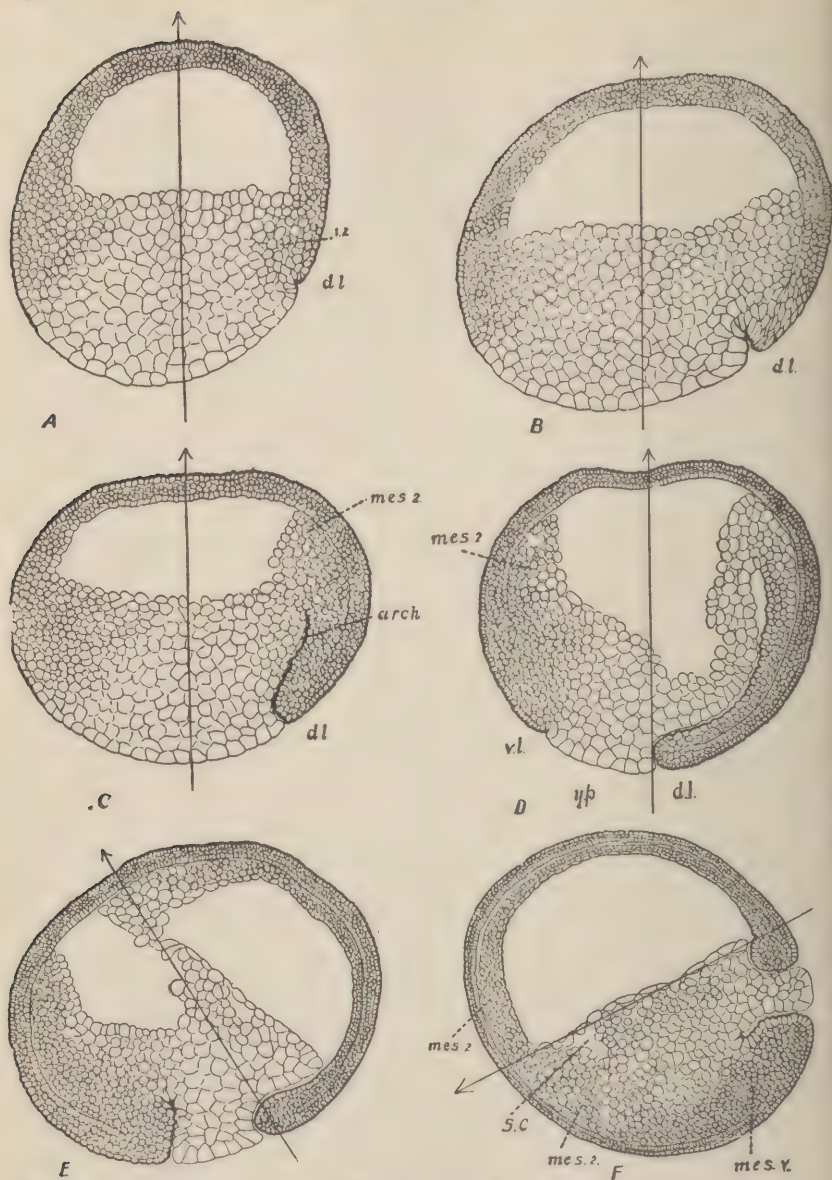
Fig. 73. — Sagittal sections through the Frog's egg during the formation and closure of the blasto-pore. From Jenkinson (Vertebrate Embryology). A-D. Before rotation. E. During rotation. F. After rotation. The arrow marks the egg-axis, its head the animal pole.

arch. Archenteron. d.l. Dorsal lip. i.z. Intermediate zone or germ ring. mes.v. Mesoderm originating at the ventral lip (i.e., a very small part of that which is classed as peristomial). mes.2. Mesoderm originating from the yolk cells pushed into the segmentation cavity (i.e., gastral). s.c. Segmentation cavity. v.l. Ventral lip. y.p. Yolk plug.

entirely clear. It seems probable, however, that it is a result of the multiplication of cells in the germ ring, which may produce an inward and upward pressure upon those inside. This also is presumably augmented by the downgrowth of this ring, tending to produce a similar inward pressure as it progresses. At all events, whatever the immediate cause, the result described above is virtually the beginning of a movement of invagination; that is, the cells of the vegetal pole of the gastrula, despite the hindrance and modifications due to their burden of yolk, are clearly tending to be pressed inward. That this is so becomes increasingly clear, moreover, as later stages are examined. Thus it will be noted (Figs. 73, 74; *C, D, E*), that although the yolk cells in the central region of the blastocœl floor apparently can not be pushed directly upward, they are, nevertheless, increasingly displaced toward the circumference.[1] The result of this is a continuation of the upward movement in these regions, until finally the displaced cells from all directions meet one another approximately at the animal pole (Figs. 73, *F;* 74, *F*). From this it should be evident that though the invagination here indicated is modified, it is not essentially different from the similarly named process in more primitive forms.

Meanwhile, as noted from the exterior, the movement of epiboly has been active, particularly at the dorsal lip, and to a decreasing extent toward either side. Thus it has happened that within that portion of the uprising (inpushing) yolk cells nearest this lip, there has extended a shallow cleft communicating with the outside at the margin of the lip. It is the rudiment of the *archenteron*, and the yolk cells which line it on every side are the *endoderm* (Figs. 73, 74; *B, C, D*). Furthermore, it will be noted that as these cells push inward and upward they do not accumulate about the inward extremity of the archenteric cavity; instead they are, as it were, pulled over its end so as to be added to its constantly extending roof (Figs. 73, 74; *D, E*). The latter is at first relatively thin, while the floor is thick. Gradually, however, the floor becomes thin — so thin indeed that in some instances it breaks through. In this event the archenteron merges with the blastocœl; in other cases the latter cavity is simply squeezed out of existence by the enlarging archenteric space. In either event, the

[1] In some instances it appears that in the earliest stage the greatest up-pushing occurs in the center of the floor, so that a vertical cross section of the cavity is at first somewhat crescent shaped. In such cases, ensuing processes, though fundamentally as described and leading to the same ultimate results, are slightly altered in detail.

final or definitive archenteric floor, subsequent to the rotation of the
gastrula which occurs at about this time, always consists of the major
mass of the yolk cells (Figs. 73, 74; F).

Involution. — There has been considerable question in the case
of the Frog as to whether the downgrowth of the blastoporal lips
is really accompanied by an inturning of the marginal cells (Fig. 74,
D, E, F, x). If such is the case, it then follows that a part of the
archenteric roof; i.e., that part nearest the lip, is composed of cells
which were derived from the outside. The argument for this view is
partly based upon the fact that some of the cells in this region of
the roof contain considerable pigment, and thus appear similar to
those upon the exterior. It has been pointed out by the opponents
of this opinion, however, that the presence of the pigment in these
cells may be due merely to the fact that they are engaged in active
division. This process it is noted is quite generally productive of
such pigment in the embryo of the Frog, and its occurrence in this
situation is not, therefore, an irrefutable argument for the exterior
origin of the cells which contain it (Brachet).

It should be clearly noted in this connection, however, that whether
or not any real involution occurs at the margin of the germ ring,
there is in this region, as repeatedly indicated, a constant downgrowth
or epiboly. Since this process, moreover, is especially active at the
dorsal lip of the ring, there is necessarily required at that point the
production of considerable material, both ectodermal and endodermal.
This material it would seem is furnished by two activities already
noted; i.e., the multiplication of cells in the ring, either outside its
margin or both outside and inside, and the confluence of this substance
to the point where the epiboly is most rapid.

Delamination. — In the general account of gastrulation in Chapter
II, it will be recalled that the origin of endoderm by the process of
splitting off was said to occur to a slight extent among the Amphibia.
It should here be stated, however, that as in the case of involution,
its occurrence is not universally admitted. Those who do describe it
(Brachet for instance), say it takes place in the following manner:

Reference to the figures will indicate that, as the process of in-
vagination begins, one of the results is as follows: As the yolk cells
(endoderm) about the margins of the blastocœl are pushed upward,
they tend to obliterate the portions of this cavity between themselves
and the ectodermal wall which bounds it. The obliteration, however,
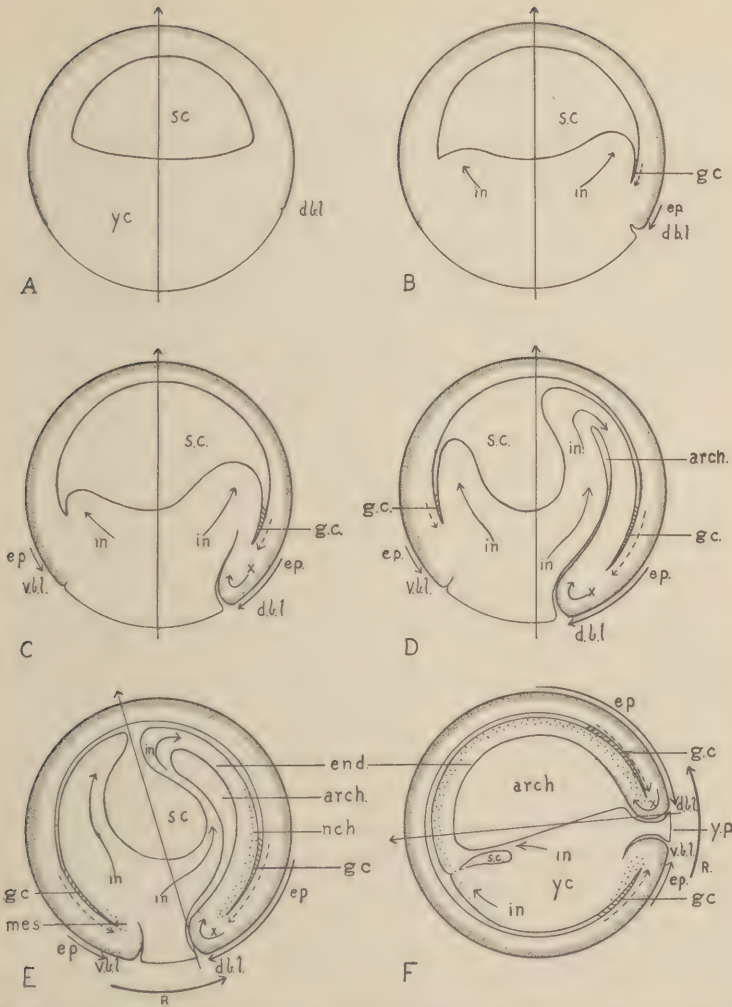
Fig. 74. — A. B. C. D. Diagrammatic representations of stages in the gastrulation of the Frog as seen in sagittal sections of the gastrula bisecting the dorsal blastoporal lip. Fine stippling indicates pigmented regions. The long straight arrow denotes the egg axis, its head marking the animal pole.

arch. Archenteron. *d.b.l.* Dorsal blastoporal lip. *end.* Endoderm. *ep.* Arrows indicating the relative amount and direction of epiboly. *g.c.* The cross hatching denotes the region of gastrular cleavage, or delamination, (according to Brachet) and the broken arrow shows the direction of its progress. *in.* Arrows indicating the direction of invagination. *mes.* Mesoderm. *nch.* Notochord (a little to either side of the mid dorsal line there would be mesoderm). *R.* Arrow indicating the direction of rotation. *s.c.* Segmentation cavity. *v.b.l.* Ventral blastoporal lip. *x.* Arrow indicating progress of possible involution. *y.c.* Yolk cells. *y.p.* Yolk plug.

is not quite complete, so that between the uprising endoderm and the *ectoderm* there remains a slight crevice. The upward extent of this crevice is then obviously increased by the continuance of the above process. By those who maintain the existence of delamination, however, it is held that besides this upward extension there is also a slight but well marked downward extension; i.e., in the direction of the blastoporal lips. This appears to occur first and most extensively in the margin of the blastocœl nearest the dorsal lip, but it presently extends entirely around the circumference. The significant point, however, is the fact that wherever the process takes place it is due apparently to a splitting apart or delamination of the cells at the bottom of the crevice (Fig. 74, *gc*). But since at all points this crevice serves to separate ectoderm from entoderm, its downward extension in the manner indicated is obviously setting apart these layers by delamination. In this particular situation this separation has also been given the name of *gastrular cleavage*.

Summary of the Processes. — To sum up the processes involved in the gastrulation of the Frog, it is found that there are at least three which were also present in Amphioxus; i.e., epiboly, concrescence, and a modified type of invagination. Besides these three there is also the probable occurrence of some involution and delamination. The last named process, if it does occur, is seen here for the first time, and, although a common method of mesoderm formation, is not again encountered in connection with gastrulation proper until we come to the Mammal.

In connection with these internal processes which accompany gastrulation it may finally be noted that there has been a considerable shifting of the yolk mass, and hence of the center of gravity. It is to these shiftings, apparently, that the rotation of the gastrula is due.

MESODERM, NOTOCHORD AND NEURAL PLATE

For the sake of clearness the process of gastrulation has been described as though it took place entirely by itself. As a matter of fact, however, such is not the case. For in the Frog, rather more than in Amphioxus, gastrulation is accompanied to a large extent by the formation of the mesoderm, the notochord, and the neural plate. We shall now turn to a description of the development of these parts.

The Mesoderm and the Notochord. — As the archenteron develops

the layer which is invaginated, involuted, or delaminated to form its roof has been referred to as being entirely endodermal. This, however, is not strictly accurate, because the cells of this roof which come next to the ectoderm are really potential *mesoderm*. Indeed, many authors refer to them at this time and even in earlier periods as actual mesoderm. At all events, the layer of the archenteric roof composed of these cells soon begins to be split off from the truly endodermal cells beneath it (Fig. 75). This delamination of the meso-

derm begins in the dorso-lateral regions, a little to either side of the median line. From thence it spreads laterally around the sides of the embryo and also anteriorly around the front. In the last named region, however, it should be noted that the layer never consists of more than loosely scattered cells. Not only does delamination occur in these directions, but presently it also begins to progress medially. For a time, however, the splits from either side do not meet. Thus there remains along the mid-dorsal line a rod of cells temporarily unseparated from the endoderm (Fig. 75, *B*). This rod is

Fig. 75. — Three stages in the differentiation of the roof of the archenteron in the Frog. From Jenkinson (Vertebrate Embryology).

arch. Archenteron. *n.ch.* Notochord. *mes.* Dorsal mesoderm.

the rudiment of the *notochord*, and a little later it is cut off, both from the sheets of mesoderm at its sides and also from the endoderm beneath (Fig. 75, *C*).

Meanwhile, somewhat below the equator, the anterior and lateral delamination of mesoderm has largely ceased. Thus in the ventral region of the embryo this layer is not formed for the most part by a

process of splitting off; instead it is completed by a downward pro-
liferation of cells from the edges of the sheets to which the dorsal
and lateral splitting has given rise.

Presently by the above means the mesoderm comes to exist through-
out the greater part of the embryo, as a separate layer between
ectoderm and endoderm. As noted, it is interrupted dorsally by the
notochord, while anteriorly the cells are very loosely arranged.
Lastly in the region of the blastopore there persists for a time an
undifferentiated mass of cells containing the elements of all three
layers. These gradually become defined, as the blastopore closes.
There now remains the question as to the ultimate or original source
of the mesoderm.

A consideration of the foregoing processes makes it at once evident
that since the mesoderm arose from the endoderm, the ultimate source
of both must have been the same. Considering first then the source of
the endoderm, it is found to have been as follows: Using the terms
anterior and posterior with reference to the definitive position of the
gastrula, it is noted that the extreme antero-dorsal, the anterior, and
the larger part of the ventral and ventro-lateral endoderm has arisen
by a modified process of invagination directly from those yolk-filled
cells which originally constituted the vegetal half of the blastula.
Most of the dorsal and dorso-lateral endoderm, on the other hand, has
originated from the material of the germ ring, and has been drawn
into place by concrescence and probably some involution. Thus upon
this basis it is clear that most of the dorsal and dorso-lateral mesoderm
(as well as the notochord) also had its source in the germ ring. But
the anterior mesoderm, and such of the ventral as did not arise by
proliferation from the dorso-lateral (or from cells in the ventral lip),
came directly from the original yolk cells.

Hence it appears that in the Frog as in Amphioxus there are two
types of mesoderm, *gastral* and *peristomial*. The portion arising
from the yolk cell endoderm is gastral from the first. That which
arises from the substance of the germ ring is, as usual, first peris-
tomial, and later gastral.

The Medullary or Neural Plate and Related Structures. — It
has already been noted that at the end of segmentation the ectoderm
of the animal hemisphere was split into an outer layer and an inner
nervous layer. During gastrulation this becomes true also in the
vegetal hemisphere. Thus toward the latter part of that process, the

double layer of ectoderm exists everywhere except in the immediate vicinity of the blastoporal lips. Throughout certain regions of the gastrula the nervous ectodermal layer then begins to thicken, the thickening being defined as the *medullary* or *neural plate*. This plate extends forward from the dorsal blastoporal lips as a median band, widening rapidly as it approaches the anterior end of the gastrula. Here it terminates, the extremity having the form of a broad curve (Fig. 76, *A*).

The thickening process which has given rise to the plate presently grows most marked around its margins, and these become slightly elevated. The elevations which thus occur along the sides of the plate are the beginnings of the *lateral neural ridges* or *folds*, while around the anterior end they are continuous with one another as the *transverse neural ridge* or *fold* (Fig. 76, *B*). Accompanying or immediately following the thickening of the nervous ectoderm which produces the ridges, there is a corresponding thinning of this layer along the mid-line of the plate. As a result there soon appears here a shallow depression. It is sometimes scarcely evident externally at this stage, but as soon as it becomes so, it is termed the *neural groove*.

THE APPEARANCE OF THE EMBRYO AT THE CONCLUSION OF GASTRULATION

Under normal conditions the above processes are usually completed in about thirty-six hours, when the general appearance of the embryo is as follows: It is still spherical in form, but has increased somewhat in size, and the medullary plate and ridges appear as described above. Furthermore, owing to the rotation which has taken place, these structures now occupy their definitive dorsal position, while the blastopore is posterior. The latter has become quite small, but is still approximately circular, and the yolk plug is slightly visible.

A COMPARISON OF GASTRULATION IN AMPHIOXUS AND THE FROG

A comparison of gastrulation, mesoderm and notochord formation, and the development of the medullary plate in Amphioxus and the Frog may now be presented in tabular form, as follows:

Gastrulation

AMPHIOXUS

The processes involved are: invagination, epiboly, involution, and concrescence.

FROG

The processes involved are: modified invagination, epiboly, concrescence, and probably some involution and delamination.

Mesoderm Formation

1. Gastrulation is virtually completed before definite setting aside of mesoderm begins.

2. The original source of the largest portion of the mesoderm is in the germ ring, and this portion is brought into position by concrescence and involution. A little at the anterior end is probably derived from the endoderm cells brought into position by simple invagination.

3. The method by which the mesoderm is set apart as such is enterocœlic evagination, or a process closely allied to it.

1. Gastrulation and mesoderm formation go on together.

2. The original source of the dorsal and dorso-lateral mesoderm is the germ ring, from which it is brought into its definitive position by concrescence and probably a little involution. The anterior, ventro-lateral, and part of the ventral, however, is largely derived directly from the yolk cells.

3. The dorsal and lateral mesoderm is set apart as such by delamination. Ventrally, however, it arises to a considerable extent by the proliferation of cells from that already formed.

The Notochord

The notochord is evaginated and pinched off from the dorsal wall of the archenteron, having had the same ultimate source as the mesoderm on either side of it.

The notochord is split off from the dorsal wall of the archenteron in the same manner as the mesoderm from which it becomes separated laterally; its ultimate source has also been the same.

The Medullary Plate and Folds

There is no split between outer and nervous ectoderm. Dorsally a median strip of ectoderm becomes slightly depressed to constitute the medullary plate. The edges of the ectoderm on each side of this plate presently become separated from the margins of the latter, and then grow together above it. The overgrowing layers so formed thus constitute only the outer half of a true medullary fold. *Later,* the margins of the plate itself also bend toward one another until they meet and fuse beneath the overgrown ectoderm.

An inner or nervous layer of ectoderm is formed by delamination over the entire gastrula. The medullary plate arises by a thickening of this layer in the mid-dorsal region. As will appear below the margins of this plate then come to constitute the crests of true neural folds. This follows from the fact that in this case the sides of the plate are carried upward and together, not *later* than, but in *company with* the ectoderm around their edges. Thus no separation occurs between the ectoderm of the plate and that surrounding it until the crests of the folds meet.

In concluding this comparison it is well once more to emphasize the fact that the above differences, at least those of gastrulation and mesoderm formation, are chiefly due to differences in relative amount of yolk. It may also be repeated that a further increase in this substance in the Fish and Bird is apparently responsible for the still greater modifications of the above processes in those animals.

THE FROG: EARLY OR EMBRYONIC DEVELOPMENT SUBSEQUENT TO GASTRULATION

The general condition of the embryo at the conclusion of gastrulation has already been indicated. Following this there occurs a period characterized by the beginning of elongation and also by the appearance of the rudiments of the main systems and organs. Thus at the end of the time in question, during which the animal has reached a length of from 2.5 to 3 mm., virtually all these rudiments are present. For this reason it will be convenient to carry forward the description of both external and internal development to about this point. We shall then be prepared to describe more clearly the remaining changes which lead to the formation of the adult.

In carrying out this plan it will not be possible to state with any accuracy the age at which a particular size and degree of development is reached, even in the same species of Frog. This is necessarily so on account of the variableness of temperature to which the eggs are subjected. It will nevertheless be helpful occasionally to mention the average age of embryos of a given condition. The student must clearly bear in mind, however, that this is never more than approximate. It is desirable to begin by considering the development of this early period in its external aspects.

EXTERNAL CHANGES

As the embryo begins to elongate certain rather conspicuous features arise as elevations or depressions of the surface. All of these structures are at first more or less connected with the medullary plate, and all of them appear at about the same time. It will be necessary, however, to describe them separately.

External Development of the Neural Tube. — The neural groove whose beginning has been noted, now becomes much deeper and more prominent (Fig. 76, C). At the same time the lateral neural ridges or

folds begin to increase their elevation and to bend toward one another until eventually their crests meet and fuse; thus is formed the neural tube. Further, as noted above, the neural plate in this case, as in that of all true Vertebrates, is involved in the process from the first. Hence no break occurs along the crests of the folds between their outer and inner layers until after these crests have met (Figs. 79; 85).[1] The phenomenon thus indicated starts somewhat anterior to the middle of the embryo in about the region of the future medulla, and from here the fusion proceeds in both directions. Anteriorly, this lateral closure is further augmented by the back growth of the transverse neural fold. Nevertheless, as will be noted presently, the completion of the process occurs later in the anterior region because of the greater space which separates the folds in this vicinity. The tube which is thus formed soon appears as a prominent ridge along the back.

The Sense Plate and the Gill Plate. — During the above process there are also developed certain other structures as follows: Just as the medullary ridges are preparing to fold in, a slight and rather narrow elevation grows outward from the antero-lateral region of each of them, and begins to extend in an antero-ventral direction. This continues until the two elevations meet one another on the front of the embryo some distance below the anterior edge of the transverse neural fold (Fig. 76, *B*). There is thus formed a relatively narrow band of slightly elevated tissue which traverses the lower anterior region of the embryo in a broad curve and then ascends on either side until it merges with the edges of the neural folds. It is termed the *sense plate*. For a time the median area between the inner edge of this semicircular band-like plate and the edge of the transverse neural fold above it remains relatively depressed; i.e., of no greater elevation than the region outside the plate. Presently, however, the distinction between this median area and the plate which constitutes its ventral and lateral boundary gradually lessens, the central region becoming almost as much elevated as its border. In this manner the sense plate comes to constitute a broad, somewhat shield-shaped region extending across the front of the embryo from side to side, while dorsally it is more or less continuous with the anterior of the neural tube (Fig. 76, *E, F*).

During the course of these processes another event is taking place

[1] It is to be noted that these literal crests of the folds are not quite identical with the "neural crests" referred to below in Figs. 79 and 85, the distinction becoming clear as the tube is about to be completed.

immediately posterior to those portions of the sense plate where it joins the neural folds upon either side. In each of these two regions there is developing another elevation which extends outward from the neural folds approximately parallel with the posterior border of the sense plate. Indeed, each of the new elevations is said by some authors to be merely a part of the original plate separated from it by the development of a depression. In any event the new raised areas, because of their future development, are termed *gill plates* (Fig. 76, *C, D, E, F*).

As the anterior portions of the neural ridges meet one another, a slight protuberance arises upon either side of the dorsal region of the sense plate (Fig. 77 *A*). These protuberances mark the out-pushings of the two optic vesicles (see below). Also at about this time there begins to develop in the middle of the sense plate a rather wide vertical groove extending from near its ventral margin dorsally to about the level of the lower edges of the optic protuberances (Fig. 77, *C*). This is the *stomodæal invagination*, the stomodæum proper, forming later at its dorsal end. It is evident that the development of this groove results in a division of the sense plate throughout the greater part of its length, so that the raised portions exist only upon either side of the median line. It may now be added that each of these raised areas constitutes the rudiment of one side of the future lower jaw or mandible, and hence each such area is designated at this time as a *mandibular arch*. Lastly, at the ventral end of each of these arches there now develops a small, somewhat elongated, and slightly pigmented depression. These depressions then deepen, while their postero-ventral ends grow toward one another and fuse, thus forming the characteristic V shaped " sucker " or *mucous gland* of the early larva.

It has been noted that the sense plate (now represented by the mandibular arches) is separated from each gill plate by a slight furrow; it remains to be added that a similar indentation also bounds each of the latter plates posteriorly (Fig. 77). Upon either side the more anterior of these furrows; i.e., the one between the mandibular arch and gill plate, marks the location of the *hyomandibular " cleft "* (in this case never an actual cleft), while the posterior one indicates the approximate position of the future *fourth branchial (gill) cleft*. There next appear upon the surface of each gill plate itself two more vertically elongated depressions denoting the beginnings of the *first* and
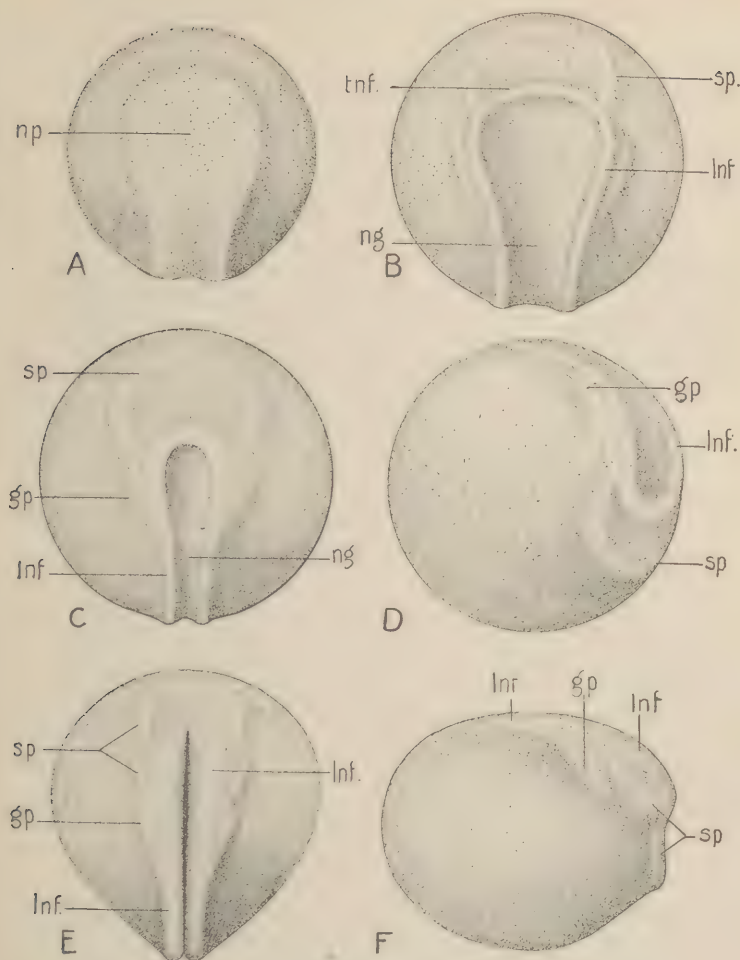
Fig. 76. — Drawings of preserved Frog embryos (*Rana pipiens*) showing successive stages in the development of the neural tube, the sense plate and the gill plates. A. Antero-dorsal view of a stage shortly after the completion of gastrulation, showing the neural or medullary plate. B. Same view of the next stage, showing the beginnings of the neural folds and the sense plate. C. Same view of somewhat later stage, showing the beginnings of the gill plates. D. Antero-lateral view of same specimen. E. Antero-dorsal view of still later stage, showing neural folds about to fuse. The sense plate and gill plates are clearly marked. F. Lateral view of same specimen.

gp. Gill plate. *lnf.* Lateral neural. or medullary folds. *ng.* Neural groove. *np.* Neural, or medullary plate. *sp.* Sense plate. *tnf.* Transverse neural or medullary fold.

second branchial clefts, the rudiment of the third branchial cleft not developing until somewhat later (Fig. 77, *D*).

It is now further obvious that between the depressions just noted the surface of each gill plate will be relatively raised so as to form ridges which are the external indications of the hyoid and branchial arches. The most anterior portion of the plate which lies between the hyomandibular cleft and the first branchial cleft is the *hyoid arch,* while the portion lying between the first and second branchial clefts is the *first branchial (gill) arch.* Since the third branchial cleft has not yet appeared, the portion of the plate posterior to the second branchial cleft really represents both the second and the third branchial arches.

The Closure of the Blastopore. — As the above events are tran-spiring anteriorly, certain processes are also occurring posteriorly, as follows: As the medullary folds begin to move toward one another, the lateral lips of the blastopore also draw together, so that the latter is no longer round. Instead it has the form of a short vertical slit (Fig. 78, *B*). Presently, moreover, these lips fuse with one another for a certain distance midway between their dorsal and ventral ends. As a result there may appear in this region for a time a slight vertical groove connecting the dorsal and ventral openings which temporarily remain. This is the *primitive streak.* In it, ectoderm, mesoderm, and endoderm meet in one mass, and from this mass, cells for all three layers are budded as the embryo increases in length. It is very im-portant to note that this primitive streak is homologous with the similarly named structures which are to be described in connection with the next two forms. It is also probably comparable with the primitive streak of Birds and Mammals. This question will be dis-cussed more fully in connection with the Chick.

The opening which remained at the ventral lip closes presently, but only the ectoderm and endoderm are involved. Hence the wall is thin at this point, and a slight pit remains. It is the *proctodæum* (Fig. 78, *D*). The dorsal opening of the blastopore persists for a somewhat longer time. It disappears externally, however, because the neural folds which extend on either side of it fuse at this point as elsewhere, and thus roof it over. This process will be further noted in connection with the nervous system.

OTHER CHANGES

Besides the features already mentioned there are a few other external alterations which usually become apparent by the time the embryo is from 2.5 to 3 mm. in length. In the first place, in connection with its slight elongation, the animal has begun to lose its spherical form, so that the convexly curved line of the back (Fig. 76, F) be-
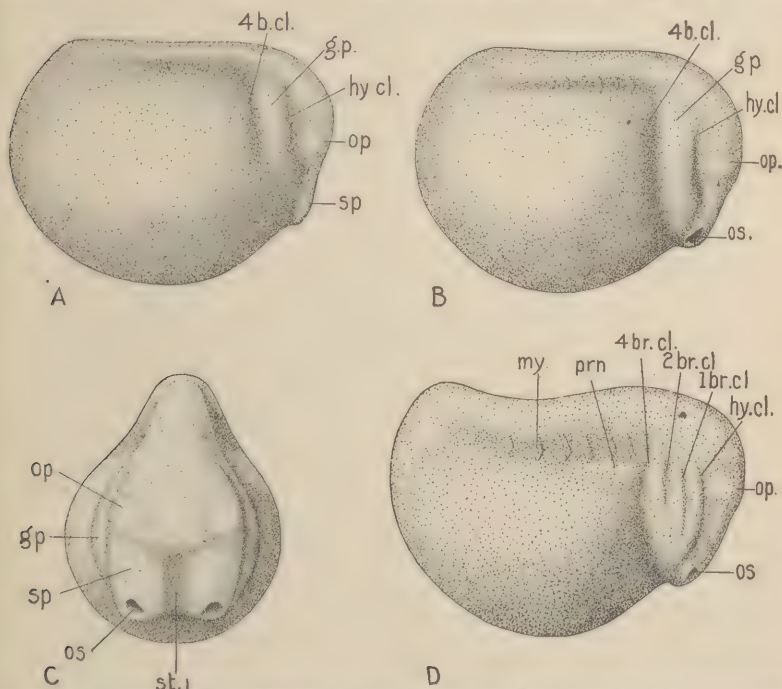
Fig. 77. — Drawings of preserved Frog embryos (*Rana pipiens*) from 2.2 to 2.5 mm. in length, showing particularly the changes in the sense and gill plates. A. Right side of a 2.2 mm. embryo. The outpushing of the optic vesicle is just beginning to appear on the dorsal part of the sense plate. The latter is becoming more clearly separated from the gill plate by the rudiment of the hyomandibular cleft, is also becoming more evident. B. Right side of a slightly older embryo than A. The invagination of the left oral "sucker" (mucous gland) is visible near the ventral end of the sense plate. C. The same embryo viewed directly from the anterior end. The stomodæal invagination and the two parts of the developing mucous gland are clearly shown. D. A 2.5 mm. embryo from the right side. The rudiments of the first and second branchial clefts have appeared upon the sense plate. Also just posterior to the dorsal part of the gill plate the outpushing due to the pronephros is visible, and the external indications of some of the myotomes are beginning to appear.

1 br. cl. 2 br. cl. 4 br. cl. Rudiments of the first, second and fourth branchial (gill) clefts. The arch anterior to each cleft is named in the text. *gp*. Gill plate. *hy.cl*. Rudiment of hyomandibular cleft. *my*. External indication of one of the myotomes. *op*. External indication of the outpushing optic vesicle in the upper region of the sense plate. *os*. Rudiment of oral "sucker" or mucous gland in the lower region of the sense plate. *prn*. External indication of the pronephros. *sp*. The sense plate, whose lower portion really represents the mandibular arch. *st. i*. The stomodæal invagination.

comes first straight and then actually concave (Fig. 77). Secondly, just posterior to the dorsal region of the gill plate there may often be noted a slight swelling, the outward indication of the internal growth of the *pronephros* or embryonic (head) kidney (see below). Also along the dorso-lateral region posterior to the gill arches and just above the level of the pronephros, > shaped markings arise giving external evidence of the *myotomes*. Lastly the embryo by this time is partially covered by cilia whose motion causes it to rotate slowly within its membranes.

Under average outdoor conditions the stage thus described is generally reached at about the end of the second day after fertilization. Let us now turn to a consideration of the internal processes which have been going on during the same period.

INTERNAL CHANGES: THE NERVOUS SYSTEM

THE NEURAL TUBE AND RELATED PARTS

The Neural Tube. — This structure, as its name suggests, possesses an internal, laterally compressed canal termed the *neurocœl* or *neural canal*. From the manner of its formation, the lining of this canal is obviously the former outer ectodermal layer of the medullary plate, while the present outer wall of the tube was previously the inner or nervous layer of that plate. Thus the floor of the tube is relatively thin, since it occupies the position of the former medullary groove where the inner or nervous layer was least developed. The lateral walls, on the contrary, are thick because they are constituted of the well developed nervous layer on either side of the groove. The roof is evidently formed as the edges of the two folds meet one another and fuse, and, like the floor, it is thin as compared with the sides. As will appear below, this is due to the fact that not all of the nervous layer along the line of fusion becomes involved in that process. Finally, it should be added that as the tube is thus made complete, the meeting of the folds likewise makes continuous the ectodermal wall above it.

The Neural Crests. — As just noted, not all of the nervous layer of the medullary plate is used up in the formation of this tube. The lateral edges of the plate; i.e., the neural ridges proper, although carried up to the region of dorsal fusion are not included in the walls of the tube. Instead, these ridges of nervous tissue are partially

constricted off from the main part of the nervous layer. Each of the two ridges is thus semi-independent, and occupies a position well up in the angle between the sides of the tube and the ectoderm of the body
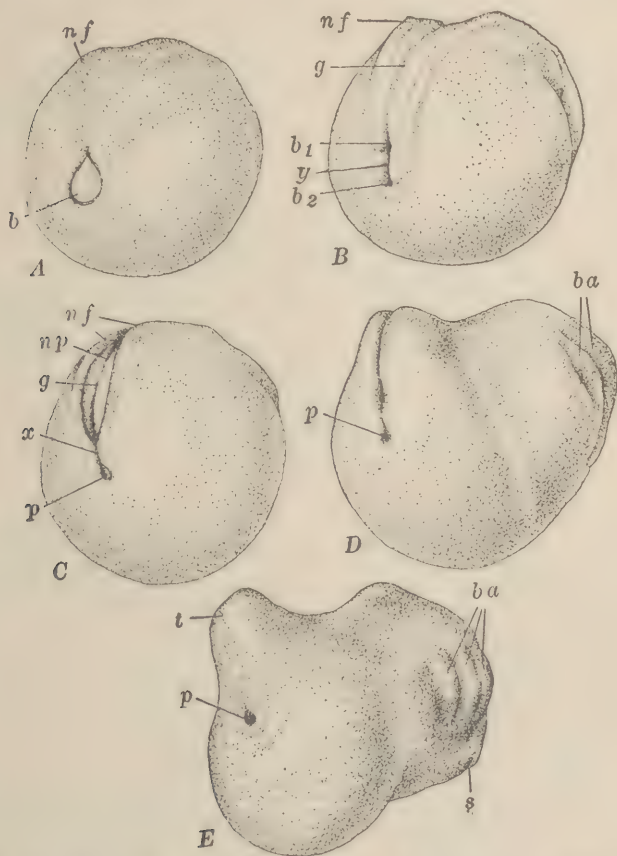
Fig. 78. — Posterior ends of a series of young Frog embryos, showing the later history of the blastopore, and the relation of the neural folds to it. The embryos are viewed obliquely from the postero-lateral aspect. From Kellicott (Chordate Development). After F. Ziegler. A. Blastopore nearly closed, neural folds just indicated. B. Blastopore becoming divided into neurenteric and proctodæal portions, lips between fusing to form primitive streak; neural folds becoming elevated. C. Neurenteric canal forming; neural folds closing together. D. Neural folds in contact throughout. E. Neural folds completely fused; tail commencing to grow out.

b. Blastopore, containing yolk plug. b_1. Rudiment of neurenteric canal (dorsal part of blastopore). b_2. Rudiment of proctodæal pit (ventral part of blastopore). ba. Branchial arches. g. Neural groove. nf. Neural folds. np. Neural plate. p. Proctodæal pit. s. Rudiment of oral " sucker." t. Rudiment of tail. x. Neural folds roofing the blastopore and establishing the neurenteric canal. y. Primitive streak.

wall (Fig. 79, *nc*). These are the *neural crests*, which presently become cut up into successive segments. Each of these segments retains a slight connection with the wall of the neural tube, and later forms the dorsal root ganglion of a cranial or spinal nerve.
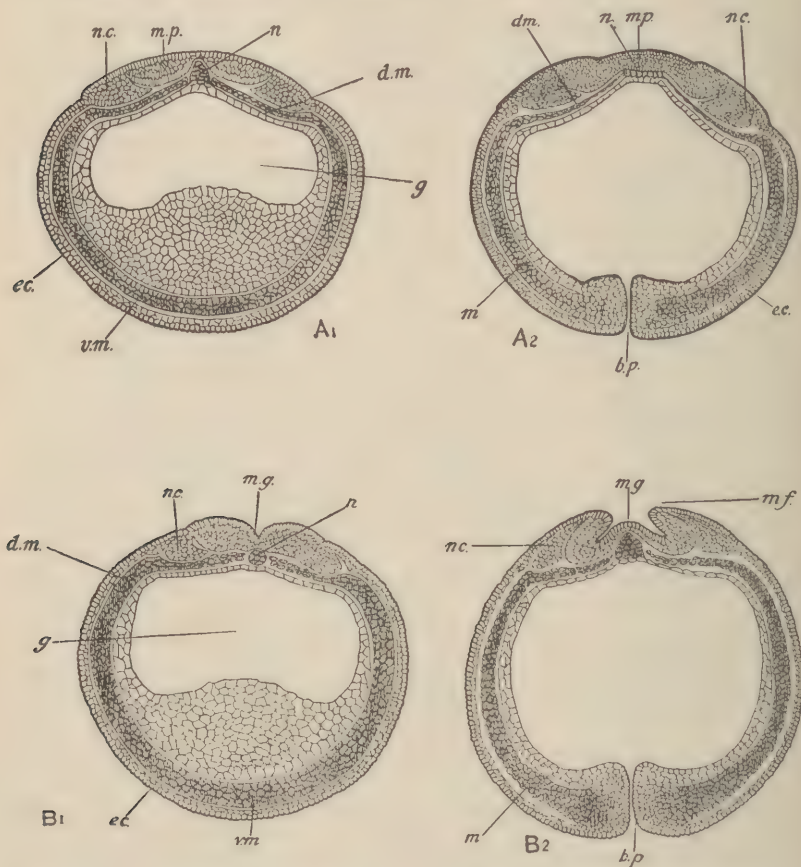
Fig. 79. — Transverse sections of the embryo of the Frog at two succeeding stages, A and B. From Jenkinson (Vertebrate Embryology). A1, B1. Sections transverse to the trunk. A2, B2. Sections transverse to the head and also, owing to the fact that the embryo is still virtually spherical, cutting the blastopore (*b.p.*) behind.

m.p. Medullary plate. *m.f.* Medullary fold. *m.g.* Medullary groove. *n.c.* Neural crest (not quite identical with the literal crests of the folds). *n.* Notochord. *m.* Mesoderm. *d.m.* Dorsal mesoderm. *v.m.* Ventral mesoderm. *ec.* Ectoderm. *g.* Gut.

THE BRAIN REGION AND SENSE ORGANS

The Brain Region. — In the anterior region the complete closure of the neural tube is somewhat delayed because of the greater breadth of the medullary plate at this point. Indeed, the process here might be still slower were it not that the growing together of the lateral edges is accompanied by the backgrowth of the transverse ridge. At
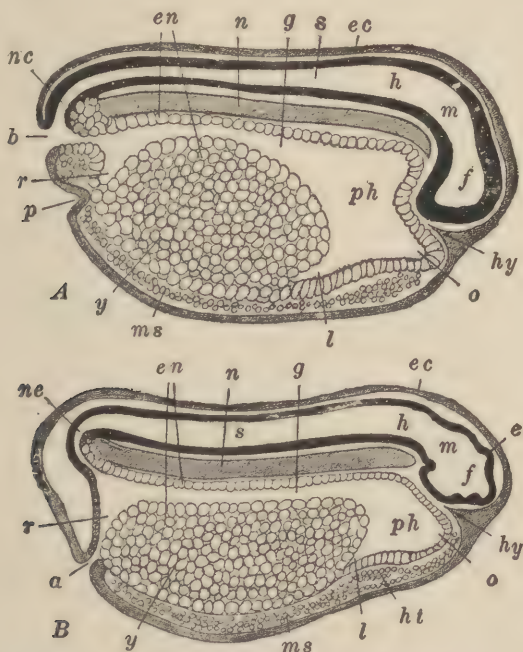
Fig. 80. — Diagrams of median sagittal sections of Frog embryos. From Kellicott (Chordate Development). After Marshall (" Vertebrate Embryology," Courtesy of Putnam's Sons). A. Just before the closure of the blastopore. B. Just after the closure of the blastopore.

a. Anal or cloacal aperture. *b.* Blastopore. *e.* Epiphysis. *ec.* Ectoderm. *en.* Endoderm. *f.* Fore-brain. *g.* Mid-gut. *h.* Hind-brain. *ht.* Rudiment of heart. *hy.* Hypophysis. *l.* Liver diverticulum. *m.* Mid-brain. *ms.* Mesoderm. *n.* Notochord. *nc.* Neurenteric canal. *o.* Oral evagination. *p.* Proctodæum. *ph.* Pharyngeal region of gut cavity. *r.* Rectum. *s.* Spinal cord. *y.* Yolk cells.

the place where this ridge and the lateral folds are about to fuse there exists for a brief time a small opening; it is the *neuropore*, and is homologous with the similar structure in Amphioxus.

At the time the medullary plate first appeared, the embryo was still virtually in the form of a sphere, and the plate followed its curvature. As the neural tube begins to form, however, the embryo, as already noted, starts to lengthen out, the line of the back becoming straight, and then slightly concave. During this process, nevertheless, the original curvature in the foremost portion of the neural tube not only persists but even increases. For this reason this part of the tube is bent downward over the front of the shorter notochord
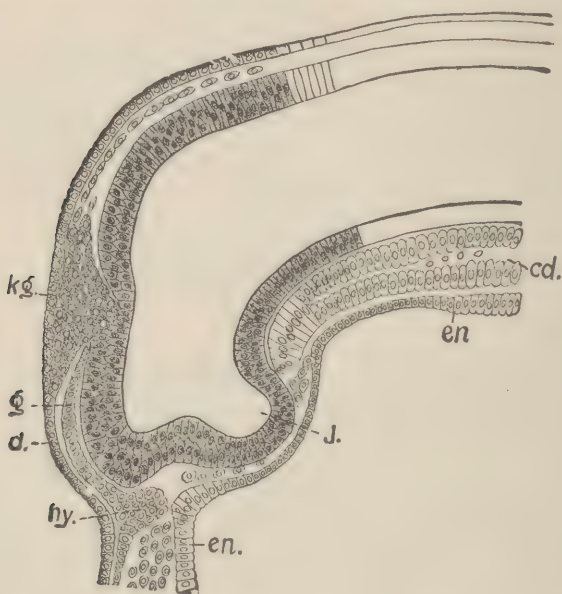
Fig. 81. — Median sagittal section through the brain of an embryo of *R. fusca* of 2.3 mm. From Von Kupffer (Hertwig's Handbuch, etc.).
cd. Notochord. *d*. Superficial layer of ectoderm (" deckschicht "). *en*. Endoderm lining of pharynx. *g*. Inner or nervous layer of ectoderm. *hy*. Hypophysis. *J*. Infundibulum. *kg*. Conical proliferation of ectoderm cells at the point of closure of the neural folds.

so that the end of the tube is lower than the level of the chord (Fig. 80). This bending is termed a *cranial flexure*. It thus happens that the roof of the tube just opposite the end of the chord contains the closing neuropore. The point of this closure is connected with the outer ectoderm for a time by a cone of cells (Fig. 81, *kg*).

Elementary Divisions of the Brain. — The constrictions which

divide the brains of most vertebrate embryos into fore-brain, mid-brain, and hind-brain have barely become evident in a 2.5 mm. Frog larva. These divisions of the brain may be determined at this time, however, by reference to two thickenings which have appeared. The first is the *tuberculum posterius,* and develops on the posterior wall of the bent portion of the tube just opposite the end of the notochord (Fig. 82). It is thus also just opposite to the point on the anterior wall at which the neuropore closed. The second is the *dorsal thickening,* which develops on the anterior wall slightly above the level of the notochord, and thus above the recent neuropore. Using these points as places of reference, the brain may now be divided into its three fundamental regions:

I. The *fore-brain* or *proscencephalon* extends from the anterior extremity of the tube; i.e., the lowest part of the bent region, to a plane joining the tuberculum posterius with a point slightly in front of the dorsal thickening.

II. The *mid-brain* or *mesencephalon* extends from the posterior boundary of the proscencephalon to another plane which joins the tuberculum posterius with a point slightly back of the dorsal thickening.

III. The *hind-brain* or *rhombencephalon* extends from the posterior boundary of the mesencephalon insensibly into the spinal cord.

It is thus evident, as indicated above, that the fore-brain is chiefly below and in front of the end of the notochord, the mid-brain is antero-dorsal to the end of the notochord, while the hind-brain lies entirely over the notochord.

Structures of Fore-brain. — Not only are the above divisions of the brain thus determined during the period which is being described, but within the foremost of these divisions there also appear the following structures:

The *infundibulum* develops as a backward outpushing of the fore-brain. It thus comes to lie beneath the anterior tip of the notochord. Immediately in front of this outpushing and thus at almost the lowest point in the fore-brain, a thickening occurs which is the rudiment of the *optic chiasma.* On the anterior side of this thickening is an evagination which leads laterally into the optic stalks (see below), and is called the *recessus opticus.* This evagination is bounded anteriorly by still another thickening, the *torus transversus.* On the anterior (morphologically dorsal) wall at about the level of the noto-

chord, another evagination appears; it is the rudiment of the *epiphysis* (Fig. 82).

There also arises at this time the rudiment of another body which is generally considered a part of the brain, though not at first connected with it. This is the *hypophysis* or *pituitary body*. It appears at this stage as a tongue of ectodermal cells extending dorso-posteriorly from the dorsal margin of the stomodæal invagination. It lies therefore just beneath the fore-brain, and is growing backward in such a way as eventually to meet the infundibulum (Fig. 82).
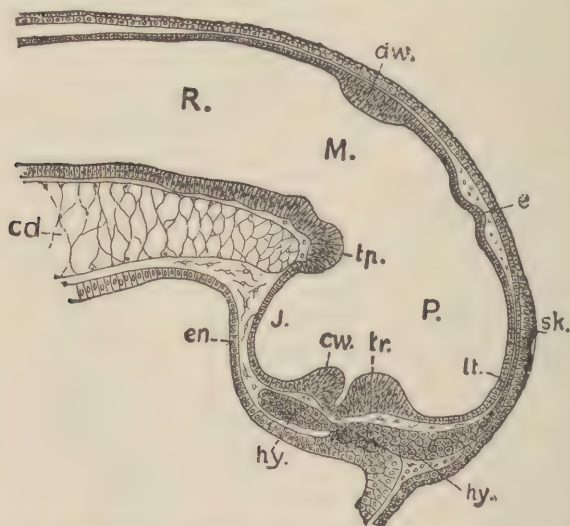
Fig. 82. — Median sagittal section through the brain of an embryo of *R. fusca*, of 2.3 mm. length, but in a more advanced stage than that of Fig. 81. From Von Kupffer (Hertwig's Handbuch, etc.).

cd. Notochord. *cw*. Rudiment of optic chiasma. *dw*. Dorsal thickening. *e*. Rudiment of epiphysis. *en*. Endodermal lining of pharynx. *hy*. Hypophysis. *J*. Infundibulum. *lt*. Lamina terminalis. *M*. Mesencephalon. *P*. Prosencephalon. *R*. Rhombencephalon. *sk*. Rudiment of olfactory placode. *tp*. Tuberculum posterius. *tr*. Torus transversus.

The Sense Organs. — Before the anterior or brain region of the medullary plate has closed, there appears on either side a patch of pigmented cells (Fig. 83). As a result of the closing process, these patches presently come to occupy positions on opposite sides of the interior of the fore-brain. The area of the brain wall including and immediately surrounding each patch now begins to push out or evaginate toward the external ectoderm of the head (Fig. 88, *A*).

These evaginations are the *optic vesicles*. Presently each vesicle reaches the ectoderm in the dorso-lateral region of the sense plate, and by its pressure here soon causes a slight external protuberance noted above. Meanwhile the regions of the vesicles nearest the brain begin to become slightly constricted to form the *optic stalks* (Fig. 88, *B*).

The ears, unlike the above parts of the eyes, do not develop from any portion of the brain itself. Instead they arise from the dorso-lateral walls of the head. The rudiment of each appears during this period as a thickened patch of the nervous layer of ectoderm opposite the hind-brain. These thickenings in part constitute the *auditory placodes* (see below under ear).
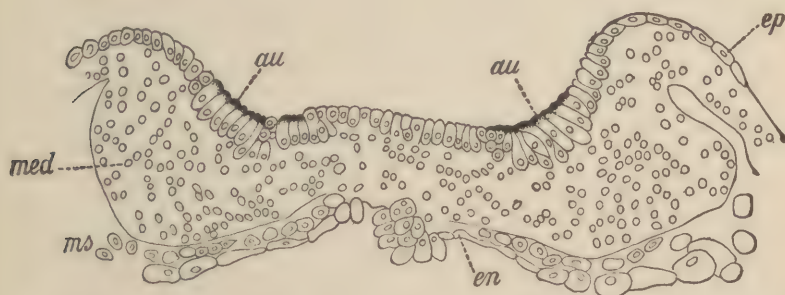
Fig. 83. — Transverse section through the anterior part of the medullary plate of an embryo of *R. palustris*, in which the medullary ridges are just forming. From Froriep (Hertwig's Handbuch, etc.). After Eycleshymer.
au. Optic patches. *en.* Endoderm. *ep.* Ectoderm (epidermis). *med.* Medullary plate. *ms.* Mesoderm.

At about the same time in another region of the head two other thickenings of the nervous ectoderm develop. In this case each is within the area of the sense plate a short space beneath and median to the corresponding optic protuberance. These are the beginnings of the olfactory organs, and are termed the *olfactory placodes* (Fig. 84, *r*). Though later each is indicated externally by a pit, these markings are usually not in evidence at this stage (see below).

THE NEURENTERIC CANAL

While the above developments have been taking place in connection with the anterior end of the nervous system there has also been a change posteriorly. It was noted in describing the externals that as

the neural folds close in this region, they roof over the dorsal part of the blastopore. As stated, however, this portion of the blastopore, though no longer communicating with the outside, still remains open.
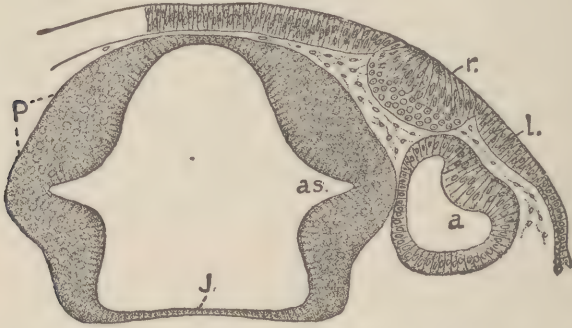
Fig. 84. — Frontal section through the fore-brain, optic vesicle, and olfactory placode (r) of an embryo of *R. fusca,* in which the tail is just growing out. From Von Kupffer (Hertwig's Handbuch, etc.).

a. Optic vesicle. *as.* Opening of optic stalk out of fore-brain. *J.* Posterior wall of infundibulum. *l.* Rudiment of lens (placode). *P.* Wall of prosencephalon. *r.* Rudiment of olfactory organ (placode).

It thus constitutes a temporary connection between the enteron and the neurocœl. As in Amphioxus, this connection is termed the *neurenteric canal* (Fig. 80).

INTERNAL CHANGES: THE ENTERON

The Fore-Gut

The anterior region of the archenteron is enlarged and lies in front of the mass of yolk cells which form the floor of the middle region. This anterior portion is therefore termed the *fore-gut,* and a little later will be differentiated into the pharynx, œsophagus, and stomach. These divisions are as yet scarcely distinguishable. Nevertheless, during the period under discussion, the fore-gut as a whole gives rise to certain rudiments as follows:

The Pharyngeal Region. — In the antero-ventral region beneath the fore-brain there is an outpocketing in the direction of the invaginated ectoderm, though the two walls are not yet in contact. It is called the *oral evagination* and may be considered as the extreme

anterior end of the *pharynx* (Fig. 80). Immediately posterior to this in the region of the fore-gut which is destined to become the pharynx proper there have already been noted the external rudiments of certain of the visceral clefts; i.e., the hyomandibular, and the first, second, and fourth branchials. Considering now the internal development of this region at a corresponding stage, the following condition is to be observed. Opposite the invaginating ectoderm which marks externally the rudiments of the above mentioned clefts the endoderm of the pharynx is beginning to push outward upon either side to form the corresponding pairs of *hyomandibular*, and *first* and *second branchial* or *gill pouches*. It should further be added that although these vertically elongated pharyngeal evaginations are called pouches, they do not actually appear as such. This is because the anterior and posterior walls of each outpushing are at this time fused together, so that no pouch cavity really exists. Thus each pouch resembles rather a two layered sheet of endoderm, extending from the fore-gut toward the ectoderm (Fig. 101).

The Liver. — In the extreme ventro-posterior part of the general pharyngeal region there is evident a slight posteriorly directed pocket beneath the anterior end of the yolk mass. This represents the rudiment of the *liver* (Fig. 80).

THE MID-GUT

The portion of the enteron following the fore-gut lies, as noted, above the main mass of the yolk cells which thus form its floor. Its lumen is relatively small with a thin roof, and sides which thicken ventrally. It is the *mid-gut*, and is destined later to develop into the intestine.

THE HIND-GUT

Posterior to the mid-gut just in front of the neurenteric canal the enteron enlarges slightly. This region is termed the *hind-gut*, and is destined to form the rectum.

INTERNAL CHANGES: THE NOTOCHORD

Within the notochord the cells become vacuolated, and intercellular vacuoles also appear. At the same time around the notochord there develop two sheaths. The outermost, known as the *primary* or

elastic sheath, is formed from the most superficial chorda cells. The *secondary* or *fibrous sheath* lies within the latter and is formed of the chorda epithelium.

THE MESODERM AND RELATED STRUCTURES

At the close of gastrulation the condition of the mesoderm is as follows: Ventrally and laterally it exists as a continuous sheet extending up to the notochord on either side. In the head and most of the pharyngeal region it is represented only by scattered cells, while posteriorly it reaches to the germ ring, which continues to bud it off. During the period we are now discussing the mesoderm thus indicated begins to give rise to various structures in the following manner:

THE VISCERAL ARCHES

It will be recalled that in the pharyngeal region at this time the hyomandibular and the first two pairs of branchial or gill pouches are developing as solid vertically elongated evaginations of endoderm. As these evaginations push out to the ectoderm, it is obvious that the mesoderm in the way of each will be thrust to either side. In this manner such mesoderm becomes more or less concentrated in the regions of the future visceral arches which are to alternate with the pouches. Indeed, it may at this time be said to represent their rudiments, whose external appearance has already been described, as having the form of raised areas between the incipient clefts. Thus in front of the first or hyomandibular pouch is the mesodermal rudiment of the *mandibular arch* (apparent externally as the lower portion of the sense plate upon either side of the stomodæum), while between the hyomandibular and first branchial pouch is the rudiment of the *hyoid arch*. The *first branchial arch* then follows the first branchial pouch, and the *second branchial arch* follows the second branchial pouch. Since, however, the third branchial pouch is scarcely formed as yet, the mesodermal element of the second branchial arch is not at this time very clearly distinguishable from the tissue posterior to it.

THE SEGMENTAL PLATES AND THE LATERAL PLATES

Along either side of the notochord posterior to the pharyngeal region, the mesodermal sheet thickens into a relatively narrow band which is termed the *segmental* or *vertebral plate*. The remainder of each sheet below this region is then called a *lateral plate*. Ventrally the two lateral plates are continuous with one another (Fig. 85, *A*).

Formation of the Cœlom. — In its dorsal region each lateral plate now begins to become split into two sheets. The outer sheet next to the ectoderm is the *somatic mesoderm* (somatopleure), while the
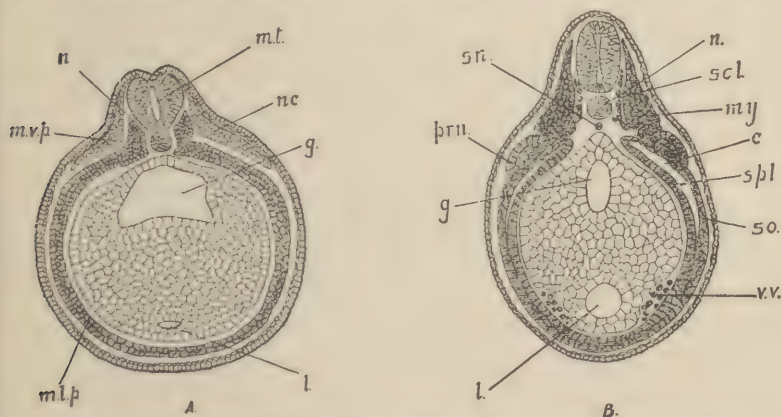
Fig. 85. — Transverse sections of Frog embryos showing the further development of the nervous system and mesoderm. From Jenkinson (Vertebrate Embryology).

m.t. Medullary tube. *n.c.* Neural crest. *n.* Notochord. *s.n.* Subnotochordal rod. *m.v.p.* Vertebral plate. *m.l.p.* Lateral plate of the mesoderm. *my.* Myotome. *scl.* Sclerotome. *c.* Cœlom (splanchnocœl). *so.* Somatopleure. *spl.* Splanchnopleure. *prn.* Pronephric ridge. *v.v.* Vitelline vein. *g.* Gut. *l.* Liver.

inner sheet next to the enteron is the *splanchnic mesoderm* (splanchopleure) (Fig. 85, *B*). Between them a space presently becomes evident which is the rudiment of the *cœlom*. Upon either side, this *cœlom* then gradually extends downward through its respective lateral plate. During the period we are describing, however, these two extensions do not reach quite far enough to meet one another beneath the gut. Thus in this region the cœlomic cavity in each plate is temporarily separated from the one on the opposite side. Besides this downgrowth of these cavities there is also an upgrowth into the mesoderm of the segmental plates (Fig. 86, *B*). Here the slight spaces which last but a brief time are termed the *myocœls*.

The Somites. — Meanwhile the segmental plates are also undergoing other changes. Just back of the pharynx each plate is being divided transversally into sections termed *somites*. During the period under consideration, about four pairs of these somites are thus formed, development proceeding posteriorly. Shortly after its formation each somite loses its connection with the lateral plate, and exists as a separate mass of cells. Within each somite so isolated the
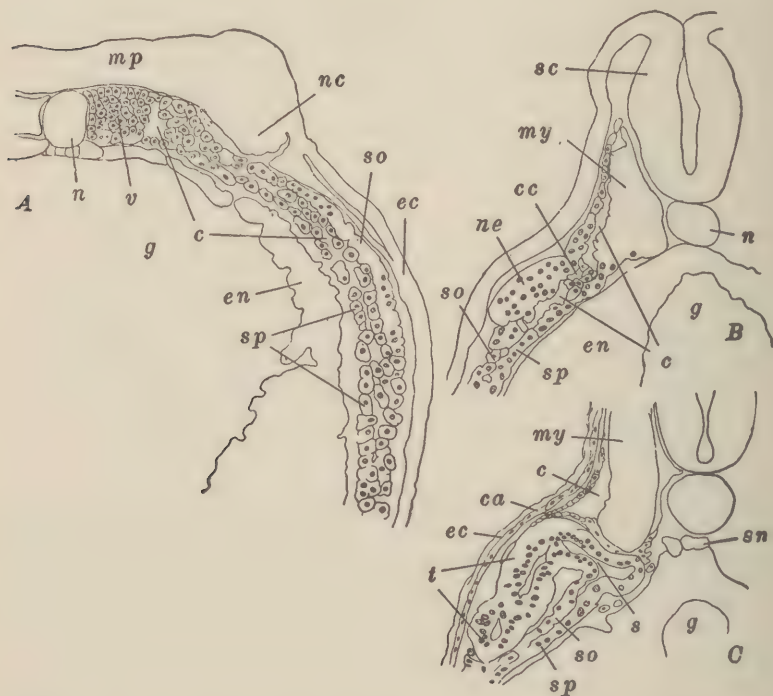
Fig. 86. — Sections through Frog embryos (*R. sylvatica*) illustrating the formation of the pronephros. From Kellicott (Chordate Development). After Field. A. Through the anterior body region of an embryo at the commencement of its elongation. B. Through the anterior end of the pronephric rudiment of an embryo in which the neural folds are just closed together. C. Through the second nephrostome of an embryo of about 3.5 mm.

c. Cœlom. *ca.* Rudiment of pronephric capsule. *cc.* Communicating canal. *ec.* Ectoderm. *en.* Endoderm. *g.* Gut cavity. *mp.* Medullary plate. *my.* myotome. *n.* Notochord. *nc.* Rudiment of neural crest. *ne.* Nephrotome. *s.* Pronephric nephrostome *sc.* Spinal cord. *sn.* Subnotochordal rod (hypochorda). *so.* Somatic layer of mesoderm (in A the reference line points to the rudiment of the pronephros). *sp.* Splanchnic layer of mesoderm. *t.* Pronephric tubule. *v.* Vertebral plate of mesoderm.

myocœl persists for a brief time, not at the center of the mass, but just beneath the outer surface. Because of its subsequent history the thin layer of cells forming this outer surface is termed the *cutis plate* or *dermatome.* For the same reason the remaining inner part of the somite is called the *myotome* (Fig. 87).

THE NEPHROTOME

Along the dorsal border of each lateral plate, just at the line of separation between lateral plate and segmental plate, is a narrow strip of somatic mesoderm which is destined to form both the larval and adult excretory systems. This strip is termed the *nephrotome,* and becomes evident as such very early (Fig. 85, *B, prn;* Fig. 86, *B*). Indeed, even before separation of the above plates this region begins to proliferate cells between itself and the ectoderm. In this way the nephrotome becomes a thick band of tissue attached along its inner border to the dorsal edge of the lateral plate, whose side it overhangs slightly, like the eave of a roof. At the very first, as segmentation appears in the vertebral plate, it also ex-

Fig. 87. — Transverse section through the sixth mesodermal somite of a 5 mm. larva of *R. temporaria*, illustrating the arrangement of the mesoderm. From Kellicott (Chordate Development). From Maurer (Hertwig's Handbuch, etc.).

c. Cutis plate. *ch.* Notochord. *D.* Gut wall *m.* Mytome (muscle plate). *me.* Nerve cord. *p.* Lateral plate. *scl.* Sclerotomal cells. *v.* Ventral process of myotome and cutis plate.

tends slightly into the nephrotomal band. Thus the single nephrotome tends to become divided into a series of nephrotomes. This division, however, is very transitory in the Frog and disappears without further

significance. As the cœlomic split begins to appear in the lateral and segmental plates, spaces also start to form in the nephrotome from about the second to the fourth somites (Fig. 86, C). This marks the beginning of the *pronephros,* the evidence of whose presence has already been noted in the description of the exterior.
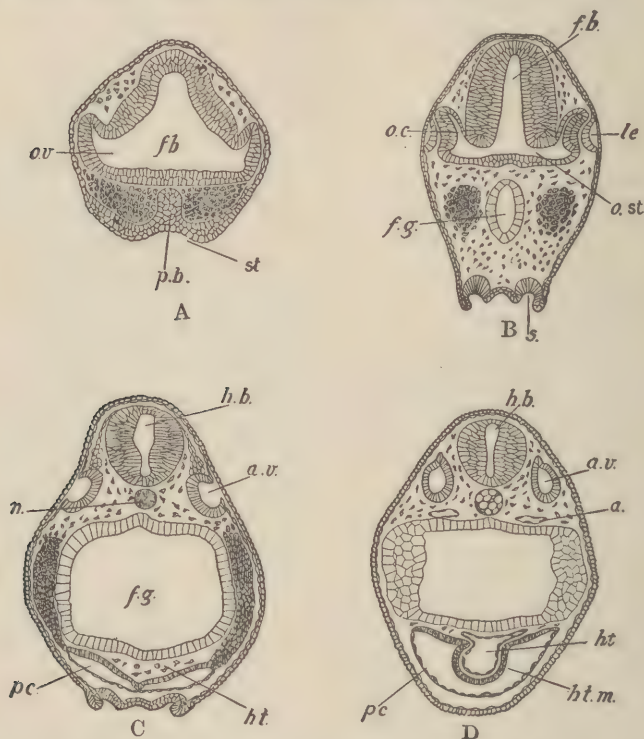
Fig. 88. — Sections illustrating the formation of the eye (A, B), ear, and heart (C, D) in the Frog. From Jenkinson (Vertebrate Embryology).

f.b. Fore-brain. *h.b.* Hind-brain. *o.v.* Optic vesicle. *o.c.* Optic cup. *o.st.* Optic stalk. *le.* Lens. *p.b.* Pituitary body. *st.* Stomodæum. *s.* "Suckers." *n.* Notochord. *a.* Aorta. *a.v.* Auditory vesicle. *ht.* Heart endothelium. *ht.m.* Muscular wall of the heart (myocardium). *pc.* Pericardium.

THE BEGINNING OF THE PERICARDIAL CAVITY AND THE HEART

In the region of the pharynx it has been indicated that laterally the rather loosely arranged mesoderm is involved in the formation of the gill arches. In the floor of this region, however, uniting the ventral

ends of these arches, there is a sheet of mesoderm coextensive pos-
teriorly with the fused lateral plates. It will be recalled that at this
period the downpushing cœlomic spaces in these plates have not reached
to the ventral side of the animal. Anteriorly, however, in the posterior
portion of the mesodermal sheet which lies beneath the pharyngeal floor,
there are now developed independently a pair of spaces (Fig. 88, C,
also Fig. 80, B). Each space lies within the sheet upon either side of
the mid-line, the two spaces being separated from one another by a
narrow median strip of the mesoderm which remains undivided (Fig.
88, C). These spaces are the rudiments of the *pericardial cavity*.

Just above the median strip, between it and the endodermal floor
of the pharynx, there also appear at this time a few scattered
cells. These cells are usually regarded as having originated like the
mesoderm of the lateral plates; i.e., by a splitting off from the en-
doderm which in this case lies above them. They are destined to
form the endothelial lining of the heart, or *endocardium* (Fig. 88, C).

THE FROG: LATER OR LARVAL DEVELOPMENT

In the last chapter the development of the embryo was discussed up to the point where it had reached a length of about 2.5 mm., and acquired the rudiments of most of the chief systems and organs. We shall now continue the history of the animal from this point to the adult condition, having regard to both the external and internal changes. The former will be considered first, under the head of three rather obvious stages which will become apparent as the description proceeds.

EXTERNAL DEVELOPMENT

Two and One-Half Millimeters to Hatching

During the first week or two, depending on the temperature, elongation progresses to a considerable extent, largely as a consequence of the outgrowth of the tail region posterior to the blastopore. Concurrent with this process, the > shaped depressions marking the boundaries of the myotomes not only become evident throughout the body region, but appear also upon the sides of the tail. At the same time just back of the gill plates the pronephric swellings increase in size. In the head the outpushings due to the optic vesicles become somewhat more pronounced, but in a slightly different position from the one which they first occupied; i.e., less upon the front of the head and more upon the side. This last mentioned change is really due to the beginning of a forward growth of the region anterior to them, which continues gradually for some time, and results in the eventual location of the eyes some distance from the tip of the snout. Meanwhile the *stomodæum* proper forms at the dorsal end of the elongated stomodæal invagination, while upon each sense plate, slightly dorsal and to one side of the stomodæum, appears a small depression, the *olfactory pit*. Each gill plate, on the other hand, now develops upon its surface another slight vertical groove lying between the rudiments

of the second and fourth branchial clefts.   This new indentation is the beginning of the *third branchial cleft*, so that the positions of all four branchial clefts are now indicated (Fig. 89, *A*).   Lastly, a short time before hatching there appears upon the upper part of the first and second branchial arches of each side a small lobed outgrowth; the rudiments of two pairs of *external gills* (Fig. 89, *B*).

The embryo (6–7 mm.), which is now ready to hatch, presently wriggles its way out of the surrounding jelly.   From this time on it may be referred to as the *larva* or *tadpole*.

From Hatching to Metamorphosis

Early Larval Life. — For a few days after hatching, the young tadpole, which is a dark brownish color, lies on its side or remains attached to some convenient object by its V-shaped mucous gland. During the first part of this period the mouth is incompletely formed, and the animal is still dependent on the yolk for its nourishment. Meanwhile the two pairs of external gills develop rapidly, the original lobes of each gill putting forth several longer minor lobes or filaments (Fig. 89, *C*).   There furthermore arises upon each third branchial arch a rudimentary third gill.   This gill, however, never develops far, and is overlapped and concealed by those anterior to it.   Aside from these features it will also be noted that the body and particularly the tail have increased in length, while the optic protuberances are still further back, as a result of the continued outgrowth of the snout.   Upon the center of each of these protuberances, moreover, there frequently appears at this time a slight depression marking the external beginnings of the actual eyes which are soon clearly visible.

In another week or somewhat less (9–10 mm.), certain further changes occur as follows.   The mouth is opened and appears as a small round orifice armed with a pair of horny jaws and with lips covered by horny rasping papillae.   At the same time the above mentioned mucous gland begins to atrophy, and the larva giving up its fixed existence swims actively about in search of food.   This consists of either animal or vegetable débris which it can scrape loose with its horny jaws and lips; in captivity it will feed readily on any sort of cereal.   In connection with this change of nourishment, the digestive organs are rapidly developed so as to give the body a full rounded appearance.   This is particularly due to the great increase in the
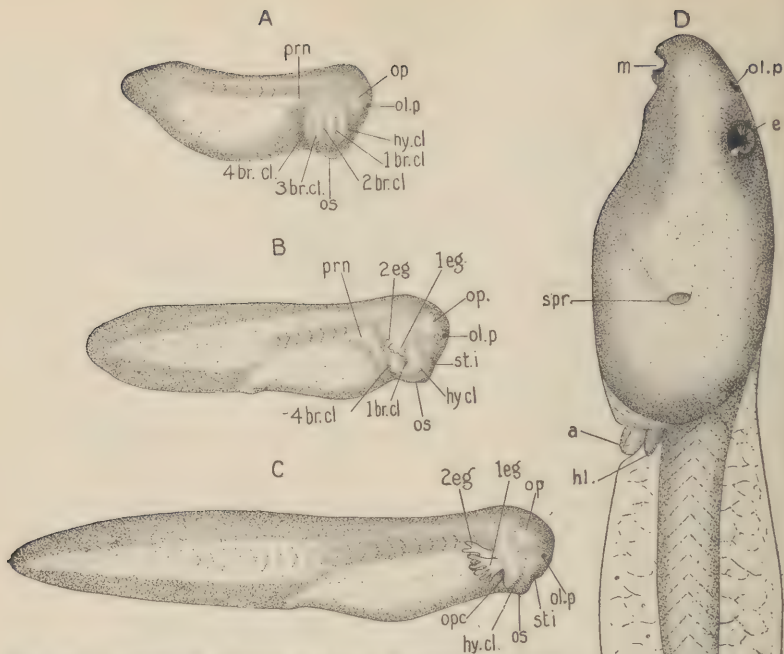
Fig. 89. — Drawings of preserved Frog embryos and larvae (*Rana pipiens*) from 4 mm. to 14.5 mm. in length. For the sake of keeping correct the relative size differences of the drawings in this figure it has been necessary to make them on a smaller scale than those in figure 77. A. Right side of a 4 mm. embryo. It will be noted that the tail has just begun to grow out, that the positions of all the visceral clefts are apparent and that the olfactory pits are present. The oral "suckers" being now entirely ventral are not actually visible from this point of view. The myotomes in this embryo and in B and C are very slightly indicated externally. B. Right side of a 6 mm. embryo. The external gills of the first and second branchial arches have begun to develop, concealing the second and third branchial clefts. The stomodæal invagination is deepening, and is slightly visible from the side. C. Right side of a 9 mm. embryo. The external gills have grown considerably, and developed several lobes. From the posterior border of the lower portion of the hyoid arch the operculum is just starting to develop, and thus covers slightly the region of the first branchial cleft. The stomodæal invagination, scarcely visible from the side, has almost given rise to the mouth. D. Left side of a 14.5 mm. larva. The external gills have been covered by the operculum, and the gill chamber opens to the outside only through the spiracle. The eye is formed, the mouth is opened into the pharynx and its lips are covered with raspers. The hind limb buds have appeared, and the tail has developed a finely veined membraneous edge or fin.

a. Anus. *1 br.cl. 2 br.cl. 3 br.cl. 4 br.cl.* Rudiments of the first, second, third and fourth branchial clefts. The corresponding arches and their positions are indicated in the text. *e*. Eye. *1 eg. 2 eg.* First and second external gills. *hl*. Hind limb buds. *hy.cl*. Rudiment of hyomandibular cleft. *m*. Mouth. *ol.p*. Olfactory pit. *op*. External indication of optic vesicle. *opc*. Edge of operculum. *os*. Oral "sucker." *prn*. External indication of the pronephros. *spr*. Spiracle. *st.i*. Stomodæal invagination.

length of the intestine which can be seen through the ventral body wall looking like a coiled spring.

As the above alterations occur in connection with the alimentary tract, certain changes also take place in the respiratory system, of which the following may be regarded as exterior. Posterior to the first and second branchial arches the incipient second and third branchial clefts become opened into the pharynx by way of the corresponding pouches as actual clefts or gill slits. The first and fourth branchial depressions then presently become true clefts in a similar manner. Concurrent with these events there is also developing from the posterior border of each hyoid arch a fold of integument called the *operculum*. These opercula then grow backward on each side, covering the gills as they progress. They also grow toward one another ventrally until they meet and fuse. Thus a closed *branchial* or *gill chamber* is formed which opens externally on the left side only, through a short funnel between the body wall and operculum, known as the *spiracle* (Fig. 89, *D*). It should finally be noted in this connection that as the closure of the branchial chamber is completed, the external gills start to atrophy and are replaced by internal gills upon the edges of the gill slits. These new organs will be more fully described in the discussion of internal changes.

Later Larval Life. — After the attainment of the above condition during the first two or three weeks of larval life, development proceeds somewhat more gradually to the time of metamorphosis. During this interval, which may last for two or three months or sometimes over the following winter, the larva increases considerably in size.[1] It also loses its brownish color and becomes more or less green dorsally, and white ventrally. Perhaps the most striking external feature, however, is the growth of the legs which begins at about the end of the first month. The fore legs develop first, but are not visible because they are covered by the operculum. The hind legs are easily seen as they arise at the base of the tail, and by the end of the second month they begin to show joints.

METAMORPHOSIS

Usually under normal conditions the tadpoles of most species begin to frequent the surface of the water during the third month. Here

[1] The larval condition is said to be prolonged by a cool season or a scarcity of food. Also the larva of certain species, e.g., the Bull Frog, *Rana catesbiana*, normally passes through the winter before metamorphosis.

they expel bubbles and gulp in air to supply the developing lungs. This is one of the signs that metamorphosis is near at hand, and at about the end of this month the final changes to the form of the adult Frog generally occur with relative rapidity.

These changes are both internal and external. The former will be described more fully later. They involve, however, a complete development of the lungs accompanied by certain changes in the circulatory system. There is also an enlargement of the stomach and liver, and at the same time a great shortening of the intestine. This change is apparently correlated with the carnivorous habits assumed by the adult. Externally the alterations are no less fundamental, and perhaps even more striking. The larval skin is cast off, and with it the horny jaws. The frilled lips likewise disappear and the mouth instead of being round becomes very wide. The tongue enlarges, and the eyes grow more prominent. The fore legs become visible by being thrust through the operculum. The left appears first because it extends through the respiratory funnel on that side, while the right is forced to break through the opercular wall. At the same time, in company with the development of the lungs, the gills dry up and the gill slits opening into the opercular chamber are closed. The hind limbs which have long been visible increase greatly in length, and the tail is rapidly absorbed. Sexual differences both internal and external now become clearly evident. There are other minor changes, but those cited comprise the more prominent and important ones.

INTERNAL DEVELOPMENT

In the description of internal changes it will be most convenient in so brief a discussion to complete entirely the history of one system before taking up the next. In the case of each, however, as many references as possible will be made to the stages noted in the account of the exterior. With this aid the student is urged to correlate as often as possible the condition reached by one group of organs with that reached by another, as well as with external changes. Only in this way is it possible to obtain a true conception of the growth of the animal as a whole.

INTERNAL DEVELOPMENT: THE NERVOUS SYSTEM

THE BRAIN

When last mentioned this organ had been somewhat artificially divided into fore, mid, and hind-brain. In the first division, furthermore, there had appeared certain structures; i.e., the infundibulum, optic chiasma, recessus opticus, torus transversus and epiphysis. Further development in the three divisions now occurs as follows:

The Prosencephalon. — At about the time of hatching there grows out from the anterior end of the fore-brain a thin walled vesicle, which represents the rudiment of the *cerebrum*. Presently its sides become thickened, and somewhat later (12 mm.), it is partially divided in two by a median longitudinal invagination of the anterior and the dorsal wall. The laterally compressed cavities of the resultant halves, or *cerebral hemispheres*, are then known as the *lateral ventricles* (Fig. 90). Posteriorly they communicate with the main cavity of the fore-brain, or *third ventricle*, by a pair of openings, the *foramina of Monro*. During the remainder of larval life the hemispheres continue to grow forward and their walls to thicken. Their anterior ends become slightly constricted away from the main portion of the hemispheres as the *olfactory lobes*. At first these are separate, but later they become fused. Thus at metamorphosis when the cerebrum is virtually mature, it comprises half of the entire brain. Furthermore, on account of this cerebral increase and the direction of the growth, the relative proportion of the parts of the brain is so altered that the cranial flexure appears to vanish. As a matter of fact, however, it is actually unchanged.

Somewhat after the first appearance of the cerebral rudiment; i.e., about 9 mm., a change occurs in the anterior-dorsal wall of the third ventricle just below and slightly in front of the epiphysis. The thin roof of this region becomes folded and hangs down into the cavity of the ventricle. Later these folds become very vascular, and are known as the *anterior choroid plexus* (Figs. 90 and 91, *B*).

With the appearance of this final structure of the prosencephalon, it is possible to further subdivide this region as follows. Suppose a plane to be passed transversely through the third ventricle from the posterior side of the choroid plexus, to the anterior side of the optic

recess. The portion of the ventricle anterior to this plane is then termed the *telencephalon,* and the portion posterior to it, the *dien-*
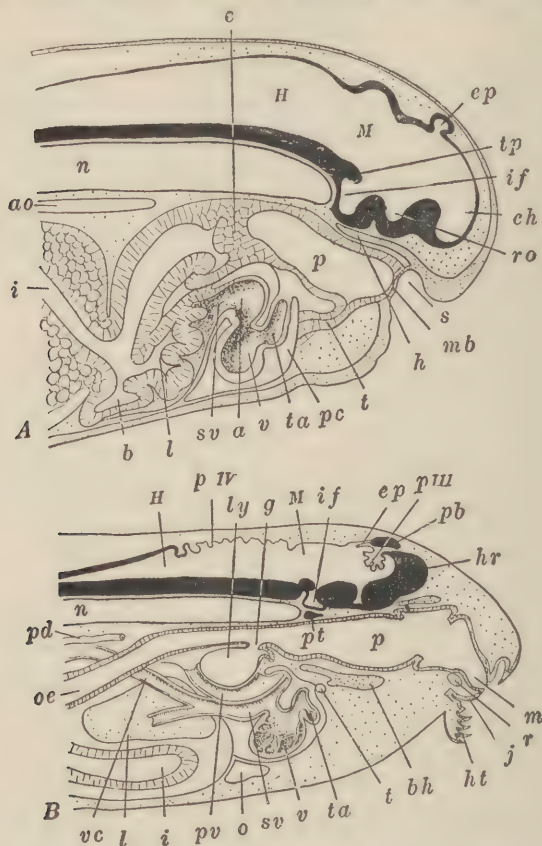
Fig. 90. — Diagrams of median sagittal sections of the anterior ends of Frog larvæ. From Kellicott (Chordate Development). After Marshall (" Vertebrate Embryology," courtesy of Putnam's Sons). A. Of a larva just before the opening of the mouth. B. Of a 12 mm. larva (at the appearance of the hind-limb buds).

a. Auricle. *ao.* Dorsal aorta. *b.* Gall bladder. *bh.* Basihyal cartilage. *ch.* Cavity of cerebral hemisphere (lateral ventricle). *e.* Epithelial plug closing the œsophagus. *ep.* Epiphysis. *g.* Glottis. *h.* Hypophysis. *H.* Hind-brain. *hr.* Cerebral hemisphere. *ht.* Horny " teeth." *i.* Intestine. *if.* Infundibulum. *j.* Lower jaw. *l.* Liver. *ly.* Laryngeal chamber. *m.* Mouth. *M.* Mid-brain. *mb.* Oral membrane (oral septum). *n.* Notochord. *o.* Median portion of opercular cavity. *oe.* Œsophagus *p.* Pharynx. *pb.* Pineal body. *pc.* Pericardial cavity. *pd.* Pronephric (more posteriorly mesonephric) duct. *pt.* Pituitary body. *pv.* Pulmonary vein. *pIII.* Choroid plexus of third ventricle. *pIV.* Choroid plexus of fourth ventricle. *r.* Rostral cartilage. *ro.* Optic recess. *s.* Stomodæum. *sv.* Sinus venosus. *t.* Thyroid body. *ta.* Truncus arteriosus. *tp.* Tuberculum posterius. *v.* Ventricle. *vc.* Inferior (posterior) vena cava.

cephalon. On this basis it is evident that both the cerebral hemispheres and the anterior choroid plexus arise from the telencephalon.

Although the pituitary body, as already noted, is not strictly a part of the brain, its further history may best be described at this point. Its backward growth continues and, at about the time the choroid plexus appears, it loses its connection with the stomodæal ectoderm. At the same time it acquires a cavity, and presently becomes united with the infundibulum. Its posterior portion eventually becomes convoluted and tubular (Fig. 91).

The Mesencephalon. — The structures of the mesencephalon or mid-brain are not so numerous as are those of the fore-brain. Its chief features are the *crura cerebri* and the *optic lobes.* The former arise gradually as a pair of ventro-lateral thickenings composed of nerve fibers connecting this portion of the brain with the fore-brain. The latter; i.e., the optic lobes, appear at about 9 mm. as a pair of swellings in the dorso-lateral region of the roof. They attain their full size at about the time of metamorphosis. The cavity of the mid-brain serves to connect the cavities of the fore and hind-brains, and is termed the *aqueduct of Sylvius.*

The Rhombencephalon. — The rhombencephalon or hind-brain is also known as the *medulla oblongata.* It lies entirely above the notochord, and is separated from the mid-brain by a slight constriction. The roof is thin, but at about the time the mouth opens (9 mm.), there appears at its extreme anterior end a small transverse thickening. This is the *cerebellum.* It later increases somewhat in size, but is always very inconspicuous in the Frog (Fig. 91).

The remainder of the roof of the medulla continues to be very thin, but at the same time that the cerebellum starts to develop it begins to become folded. Soon blood vessels extend down into these folds, and thus is formed the *posterior choroid plexus* (Fig. 90, *B*). The floor and the ventro-lateral walls of the hind-brain become thickened as nerve tracts. Its cavity connecting anteriorly by way of the aqueduct of Sylvius with the third ventricle, and posteriorly with the neural canal, is called the *fourth ventricle.*

The Spinal Cord. — Posterior to the brain region the neural tube gradually assumes the character of the adult spinal cord. The laterally compressed neural canal is, as already noted, lined by cells which were originally external. These are non-nervous and ciliated, and are known as *ependymal cells.* The relatively thick nervous

layer which constitutes the bulk of the lateral walls gives rise to both supporting or *glia* cells, and to *neuroblasts* or primitive nerve cells. The latter lie relatively near the central canal, and comprise the so-
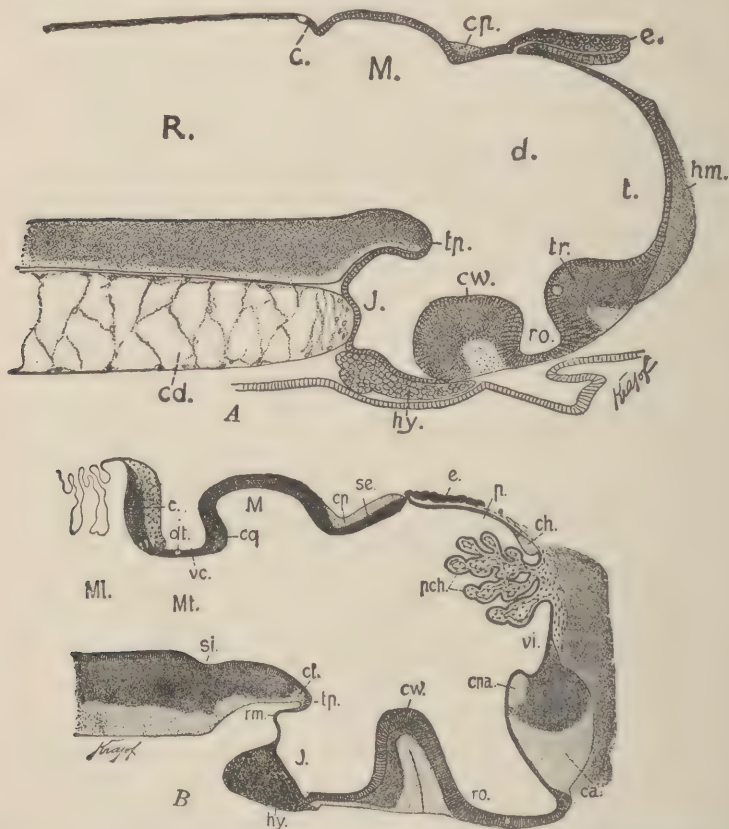
Fig. 91. — Median sagittal sections through the brain of the Frog. From Von Kupffer (Hertwig's Handbuch, etc.). A. Of a larva of *R. fusca* of 7 mm. in which the mouth was open. B. *R. esculenta* at the end of metamorphosis.

c. Cerebellum. *ca.* Anterior commissure. *cd.* Notochord. *ch.* Habenular commissure. *cp.* Posterior commissure. *cpa.* Anterior pallial commissure. *cq.* Posterior corpus quadrigeminum. *ct.* Tubercular commissure. *cw.* Optic chiasma. *d.* Diencephalon. *dt.* Tract of IV cranial nerve. *e.* Epiphysis. *hm.* Cerebral hemisphere. *hy.* Hypophysis (pituitary body). *J.* Infundibulum. *M.* Mesencephalon. *Ml.* Myelencephalon. *Mt.* Metencephalon. *p.* Antero-dorsal extension of diencephalon. *pch.* Choroid plexus of third ventricle. *R.* Rhombencephalon. *rm.* Recessus mammillaris. *ro.* Optic recess. *se.* Roof diencephalon. *t.* Telencephalon. *tp.* Tuberculum posterius. *tr.* Torus transversus (telencephali). *vc.* Valvula cerebelli. *vi.* Ventriculus impar (telencephali) (third ventricle).

called *gray matter*. The fibers which arise from them, however, course up and down through the more superficial parts of the cord, helping still further to thicken it, and constituting the *white matter*.

This thickening occurs first in the dorsal-lateral regions, thereby causing the neural canal to lie temporarily very near to the ventral side (Fig. 92, *A*). Gradually, however, the growth of cells and fibers spreads downward so that eventually the canal lies practically in the
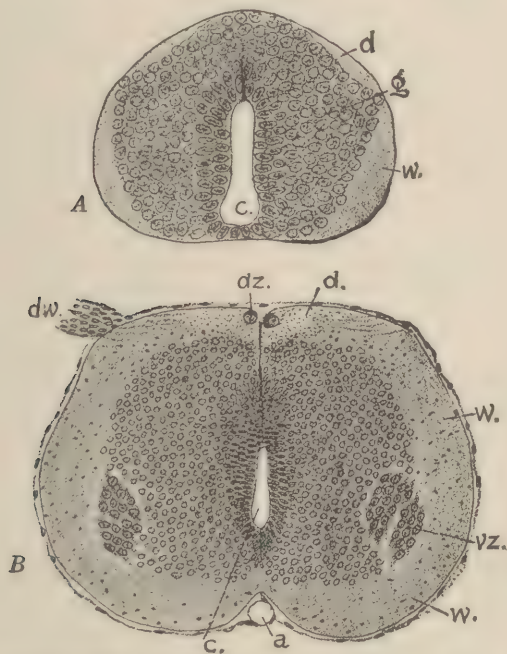
Fig. **92.** — Transverse sections through the spinal cord of *R. fusca*. From Von Kupffer (Hertwig's Handbuch, etc.). A. Through the anal region of a larva of **7 mm.** B. Through the anterior body region of a larva during metamorphosis.
a. Spinal artery. *c*. Central (neural) canal. *d*. Dorsal column (white matter). *dw*. Dorsal root of spinal nerve. *dz*. Atrophied dorsal cells. *g*. Gray matter. *vz*. Ventral cells. *w*. Dorso-lateral and ventro-lateral column (white matter).

middle of the cord. The ventro-lateral growth, moreover, is slightly greater than that exactly along the mid-ventral line. Thus a shallow depression occurs here in which runs the spinal artery (Fig. 92, *B*).

Posteriorly the neurenteric canal becomes severed even before hatching, and the nerve cord continues straight out into the tail. This portion of the cord is of course lost at metamorphosis.

The Peripheral Nervous System

The Cranial Nerves. — In discussing nerves in general, it is quite customary to divide them into *afferent* or sensory nerves, and *efferent* or motor nerves. In describing both the cranial and spinal nerves,
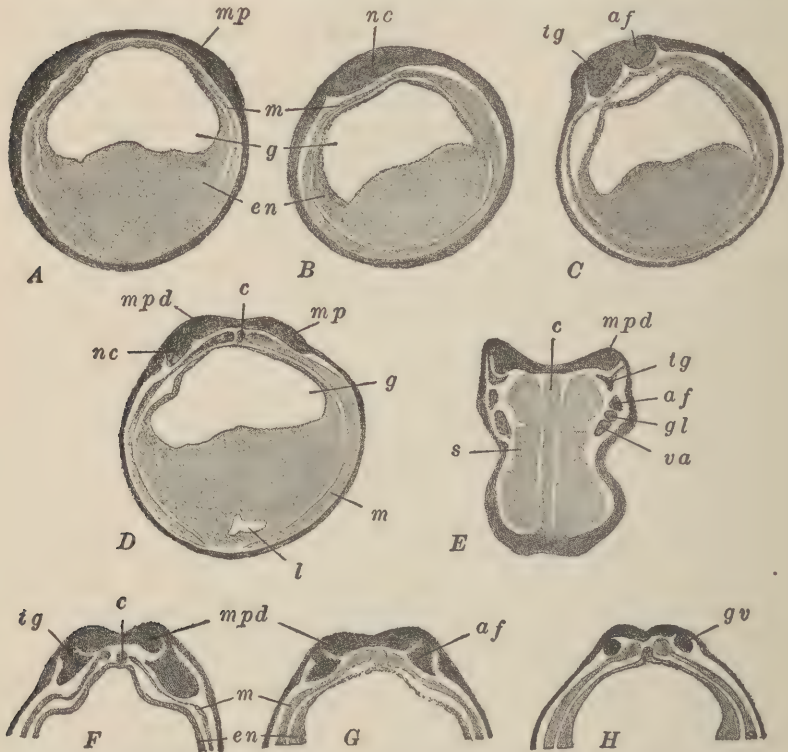
Fig. 93. — Sections through young Frog embryos (*R. fusca*), illustrating the development of the crest segments ("ganglia") and placodes. From Kellicott (Chordate Development). After Brachet. A. Transverse section through the neural plate of an embryo before elongation begins. B. Sagittal section to one side of the mid-line, through an embryo of the same age as A. C. Sagittal section, to one side of the mid-line, through an embryo just beginning to elongate. D. Transverse section through an embryo slightly older than that of A and B. E. Frontal section through an embryo with three or four pairs of mesodermal somites. F, G, H, Three transverse sections through an embryo just beginning to elongate (same age as C), showing the trigeminal, acustico-facial and glossopharyngeal-vagus crest segments ("ganglia").

af. Acustico-facialis crest segment ("ganglion"). *c.* Notochord. *en.* Endoderm. *g.* Gut cavity. *gl.* Glossopharyngeal crest segment ("ganglion"). *gv.* Glossopharyngeal-vagus crest segment ("ganglion"). *l.* Liver diverticulum. *m.* Mesoderm. *mp.* Primitive medullary plate. *mpd.* Definitive medullary plate. *nc.* Neural crest. *s.* Mesodermal somites. *tg.* Trigeminal crest segment ("ganglion"). *va.* vagus (pneumogastric) crest segment ("ganglion").

however, it is convenient to add a third category; i.e., *mixed nerves,* which contain both afferent and efferent fibers.   It is understood that all these nerves occur in pairs, but it will be necessary to describe the development only on one side.

Purely Afferent Nerves. — There are three cranial nerves which are purely afferent; namely, the I or *olfactory nerve,* the II or *optic nerve,* and the VIII or *auditory nerve.* The first two are of a rather special nature, and are also very closely connected with the development of the sense organs which they supply. It will therefore be more convenient to describe them later in connection with those organs. The VIII nerve on the other hand arises in such close connection with the mixed nerves that it will be described under that category.

Mixed Nerves and the Auditory Nerve. The nature of the neural crests has already been indicated, and it was noted that each crest becomes divided into segments. In the brain region there are three such segments on each side of the head. Considerably before hatching (3–4 mm.), moreover the nervous layer of ectoderm on the inside of the head opposite the segments becomes thickened into patches termed *placodes,* one opposite each of the first two segments, and two opposite the last. It is then from certain nervous or ganglionic elements of these structures; i.e., the crest segments and placodes, that the ganglia of the V, VII, VIII, IX, and X nerves (Fig. 93) and their afferent fibers develop in the manner indicated below. Lastly, it may be noted that the original strands of cells attaching the crest segments to the brain (Fig. 94, *A*) serve ultimately only as the sheaths of the root nerve fibers, whose origin is to be described.

The V or *trigeminal* nerve ganglion develops from the dorsal and superficial cells (ganglionic element) of the most anterior crest segment in conjunction with cells derived from the inner or ganglionic portion of the corresponding placode (Fig. 94, *B*). The anterior part of the ganglion is derived almost entirely from the anterior portion of the placode, and gives rise to the fibers of the *ophthalmic* branch of the V nerve. The posterior part consists of both crest and placode elements, and is sometimes distinguished as the trigeminal ganglion proper, or the *Gasserian* ganglion. This part gives rise to the *mandibular* and *maxillary* branches. From both parts of the ganglion a common bundle of fibers also grows inward to the medulla constituting the sensory element of the V nerve root. The ophthalmic branch of the nerve is destined for the skin of the snout, while the mandibular
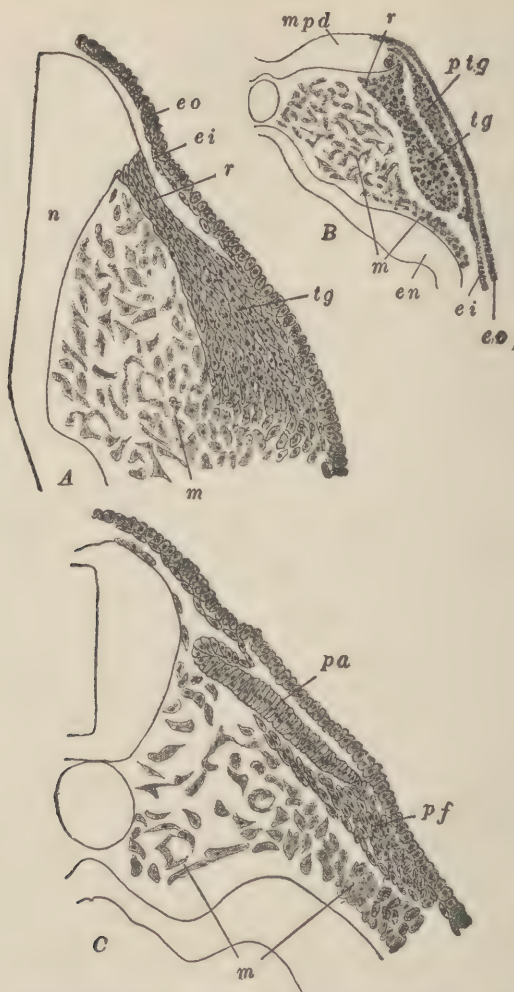
Fig. 94. — Portions of sections through the head of the Frog (*R. fusca*), illustrating the formation of the placodes and the history of the crest segments ("ganglia"). From Kellicott (Chordate Development). After Brachet. A. Transverse section through the trigeminal crest segment ("ganglion") of an embryo of 3 mm. B. Transverse section through the trigeminal crest segment ("ganglion") and placode of an embryo with three or four pairs of mesodermal somites. C. Transverse section through the facial ganglion and auditory placode of an embryo of 2.8 mm.

ei. Inner or nervous layer of ectoderm. *en*. Endoderm. *eo*. Outer layer of ectoderm. *m*. Mesoderm. *mpd*. Definitive medullary plate. *n*. Nerve cord. *pa*. Auditory placode. *pf*. Facial placode. *ptg*. Trigeminal placode. *r*. Spinal prolongation of ganglion. *tg*. Trigeminal crest segment ("ganglion").

ana maxillary branches supply the lower and upper jaw. As all of these branches start to develop previous to hatching, in a 9 mm. tadpole they are well established. It may be added that the non-nervous part of the crest segment, in this instance the major part, grows ventrally and contributes to the mesenchyme of the mandibular arch. The superficial (outer) non-nervous part of the placode, on the other hand, disappears.

The VII or *facial* nerve ganglion develops from the ganglionic part of the second (*acustico-facialis*) crest segment, together with a portion of the ganglionic part of the second placode. As before, some of the fibers which issue from this ganglion proceed inward to the medulla, forming the sensory element of the root, while others grow outward as the afferent fibers of the nerve. Before hatching the latter have divided into the *hyoid* and *palatine* branches. Here also it remains to be noted that a considerable part of the crest segment is non-nervous and contributes in this case to the mesenchyme of the hyoid arch. No part of the placode in this instance, however, disappears. One portion is utilized as just described, while the remainder goes to form the ganglion of the VIII nerve and the auditory apparatus, as indicated below.

The IX and X or *glossopharyngeal* and *vagus* (pneumogastric) ganglia both arise from the ganglionic portion of the last cranial crest segment in conjunction with the inner; i.e., ganglionic part of the third and fourth placodes respectively. The IX, however, has in it a much larger portion of the placode element than does the X. Fibers from these two ganglia enter the medulla as a single root. Peripheral outgrowths from the IX ganglion supply the first branchial arch, while branches from the X pass to the remaining branchial arches. The vagus ganglion also sends branches to the viscera and to the lateral line organs (see below). Both of these ganglia with their nerves develop quite early, and in a 9 mm. larva all the main branches of the vagus nerve are present. In this case the non-nervous part of the crest segment is not large, but, so far as it exists, it goes to form mesenchyme. The superficial non-nervous portions of the placodes disappear.

It may now be added that the efferent fibers (axones) for each of these four nerves (V, VII, IX, and X) grow out from neuroblasts in the walls of the medulla. They pass out of the brain along with the sensory root fibers of the respective ganglia, and are distributed with those branches of the nerves which pass posterior to the visceral clefts.

The VIII or *auditory nerve* is, as already noted, entirely sensory, and its ganglion arises from the ganglionic portion of that part of the second placode which is not involved in the formation of the ganglion of the VII nerve. The more superficial portion of this placode as usual is not included in either the VII or VIII nerve ganglia, but nevertheless, as suggested above, it does not in this instance disappear. Instead it remains in close contact with the latter ganglion, and develops later into the so-called inner ear, as described below. Because of the prominent part which the major portion of this second placode then plays in connection with the auditory apparatus, it is frequently referred to as the *auditory placode* (Fig. 94, *C*), already noted in the account of an earlier stage. The roots of the VII and VIII nerves are indistinguishable from one another previous to the opening of the mouth (9 mm.).

Purely Efferent Nerves.—The III, IV, and VI nerves are all *motor ocular* nerves which innervate the muscles of the eye. Their development is imperfectly known, but they seem to arise from neuroblasts in the medulla. The III appears first, just before hatching, and the others slightly later.

The Spinal Nerves. — The ganglia of the spinal nerves, like those of the cranial nerves, originate from the neural crest segments, which in this region correspond to the somites. In this case, however, no placodes are present. From each crest segment, fibers grow inward and connect with the dorsal part of the cord. These are known as the *dorsal roots* of the spinal nerves (Fig. 95). At the same time other fibers grow outward to the skin, and other sensory organs; as in the head, all of these ganglion fibers are afferent.

Fig. 95. — Transverse section through 8.6 mm. larva of *R. esculenta,* illustrating the relations of the sympathetic cord and spinal nerve. From Kellicott (Chordate Development). After Held.

a. Dorsal aorta. *c.* Spinal cord. *d.* Dorsal (sensory, afferent) root of spinal nerve. *m.* Myotome. *n.* Notochord. *r.* Ramus communicans. *sc.* Sympathetic cord. *sg.* Spinal ganglion. *sn.* Spinal nerve trunk. *v.* Ventral (motor, efferent) root of spinal nerve.

While this is occurring dorsally the *ventral nerve roots* also arise (about 4 mm.). Each of these roots consists of a bundle of fibers (*axones*) originating from neuroblasts in the ventral part of the spinal cord; at or just beyond each dorsal root ganglion the fibers of the respective ventral bundle mingle in a common sheath with the outgoing fibers of the ganglion. Thus, since the ventral root fibers are all efferent, each nerve sheath containing both sorts constitutes a mixed nerve (*spinal nerve trunk*) as in the cases of a similar condition in the head. This trunk soon divides into a dorsal and a ventral branch, each of which now contains both afferent and efferent fibers; the former pass to the various sense organs and the latter to the muscles.

The Sympathetic System. — The sympathetic nervous system is thought to arise from certain cells which mostly have their origin in the spinal ganglia. These cells first appear, however, in small collections upon the spinal nerves at about the level of the dorsal aorta, a position in which they may be noted shortly before hatching. Presently they migrate to the aorta, along each side of which they give rise to a *sympathetic cord*. From these cords, nerve fibers later grow back to the spinal ganglia, as the *rami communicantes*. Still other fibers proceed to the viscera, and along these, cells migrate to form the various peripheral sympathetic ganglia.

ORGANS OF SPECIAL SENSE

The Eye. — When the rudiments of the eye were last considered the optic stalks were just beginning to be defined as such, owing to a slight constriction between the optic vesicles and the brain. This process is now rapidly completed so that the stalks are clearly indicated. It is then evident that they do not join the vesicles exactly at the centers of the latter but nearer to their ventral sides. There then begin certain changes in connection with the vesicles themselves as follows:

The wall of each vesicle next to the ectoderm is flattened and then pushed inward. By this process the cavity of the optic vesicle is obliterated, and at the same time a double-walled cup is formed, the *optic cup* (Fig. 96). It must be noted, however, that the direction of this invagination is not exactly horizontal. It begins rather in the ventro-lateral region and proceeds obliquely upward. This fact, together with the original relation of the vesicle and stalk, means that

the latter will necessarily be attached to the cup at its ventral edge. The rim of the cup now grows outward, particularly in its ventral and lateral regions, these being the regions which, as a result of the direction of invagination, are furthest from the ectoderm. This outward extension of the sides of the cup leaves between their ventral edges a slight fissure extending inward to the optic stalk. This is the *choroid fissure*, whose length is somewhat further increased by the continued outgrowth of the sides of the cup. Furthermore, concurrent with this outgrowth the entire rim begins to bend toward the center of the cup's aperture, thus obviously decreasing its diameter. This aperture, which faces the ectoderm, is the *pupil*, from whose ventral edge the choroid fissure runs back to the optic stalk.

Fig. 96. — Plastic figure of hemisected optic vesicle, lens and optic stalk of the Frog. From Kellicott (Chordate Development). *f.* Choroid fissure. *l.* Lens. *pc.* Posterior chamber of eye. *pl.* Outer or pigmented layer of optic cup. *rl.* Inner or retinal layer of optic cup. *s.* Optic stalk. *v.* Original cavity of optic vesicle.

Meanwhile, about the time of hatching, a thickened portion of the inner ectoderm on the wall of the head opposite the pupil becomes constricted off as a solid rounded mass of cells (Fig. 97). This is sometimes, though erroneously, called the visual placode. It presently acquires a central cavity, which is soon obliterated, however, by the thickening of the cells on the side toward the pupil. This mass now moves in to the center of the pupil, and becomes the *lens*.

Shortly after hatching the cells in the walls of the optic cup begin to differentiate. The inner wall thickens and develops into the *retina*, its outermost cells becoming the *rods* and *cones*. Its inner cells; i.e., those toward its cavity, however, form neuroblasts and produce fibers which pass over the surface of the retina and out of the cup through the inner end of the choroid fissure. They then grow along the outside of the ventral wall of the optic stalk to the brain. Here the fibers from opposite sides cross one another to form the *optic chiasma*. These fibers constitute the real II or *optic nerve*, though the stalk is often referred to as such. The wall of the stalk invaginates to form a canal or sheath for the fibers, thus obliterating its own inner cavity.

The outer wall of the cup forms a thin pigmented layer on the back of the retina; i.e., next to the rods and cones.
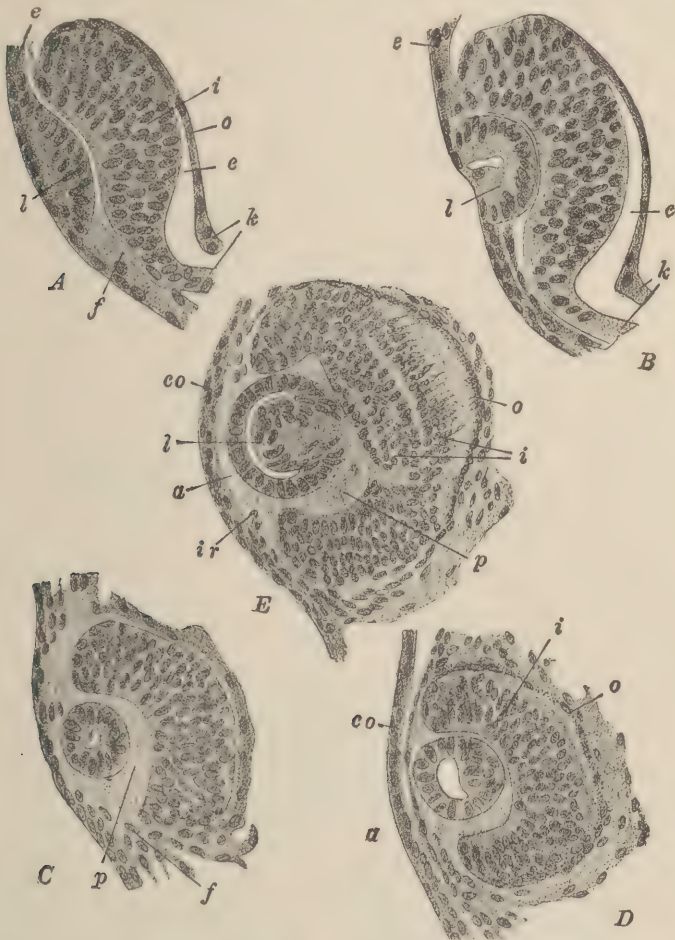
Fig. 97. — The development of the eye in the Urodele, *Siredon pisciformis.* From Kellicott (Chordate Development). After Rabl. A. Of embryo with about twenty-five pairs of somites, showing the thickening of the lens rudiment. B. Invagination of the lens and formation of the optic cup. C. Lens separating from the superficial ectoderm in an embryo of about thirty-five pairs of somites. D. Thickening of the inner wall of the lens. E. Shortly before hatching; differentiation of the rods and cones in the retinal layer.

a. Anterior chamber of eye. *c.* Cavity of primary optic vesicle. *co.* Cornea. *e.* Ectoderm of head. *f.* Choroid fissure. *i.* Inner or retinal layer of optic cup. *ir.* Rudiment of iris. *k.* Optic stalk. *l.* Lens. *o.* Outer or pigmented layer of optic cup. *p.* Posterior chamber of eye.

Slightly before hatching the lips of the choroid fissure begin to fuse, and before long the process of fusion becomes complete at every point save in the immediate vicinity of the optic stalk. Here an opening persists through which the blood vessels and nerve fibers enter the cavity of the cup. At the edge of the pupil the closure is marked by a thickening, the *choroid knot*, and from this arise the cells of the *iris*.

The *vitreous humor* is formed in the cavity of the cup by cells budded from the retinal wall and from the inner side of the lens. It is thus entirely ectodermal. The *choroid coat* of the eye is laid down outside the pigmented layer, and outside of all is the tough *sclerotic coat*. Both the choroid and sclerotic tissues are derived from mesenchyme. Opposite the lens the ectoderm of the head becomes transparent, and, again with the addition of mesenchyme, forms the *cornea*. The detailed development of the eye is not entirely completed until metamorphosis.

The Ear.

The Inner Ear or Membraneous Labyrinth. — Just before hatching the superficial part of the auditory placode; i.e., the part not involved in the formation of the VII and VIII nerve ganglia, moves in slightly from the ectoderm. At the same time it invaginates to form a closed sac or vesicle, the *auditory sac* or *otocyst*, from whose dorsal wall a small evagination appears as the rudiment of the *endolymphatic duct* (Figs. 88, *C, D,* and 98, *A, B, C*). An oblique partition now (10–12 mm.) begins to grow across the cavity of the otocyst in such a way as to divide it into an outer and ventral portion, the *saccule,* and an upper and inner portion, the *utricle*. These cavities remain connected by a small pore in the membrane (Fig. 98, *D*).

During the growth of the above partition there appear upon the inner surface of the wall of the utricular portion of the otocyst, two pairs of ridges. One pair is vertical and anterior, the other horizontal and lateral, upon the side nearest the ectoderm. Presently (15 mm.), there is added another pair which is posterior and vertical. The edges of each pair of ridges now fuse with one another along their entire length, thus giving rise in each case to a tube open at each end into the cavity of the utricle. The tubes thus formed are the rudiments of the three *semi-circular canals*. From the manner of their formation these tubes or canals evidently lie upon the inside of the utricular wall. Shortly, however, each canal pushes outward and

presently becomes constricted away from the wall of the utricle except at its ends. The canals which thus come to lie outside of the utricle now continue to grow, and so reach the adult condition. During this latter process, however, each canal acquires an enlargement at one of
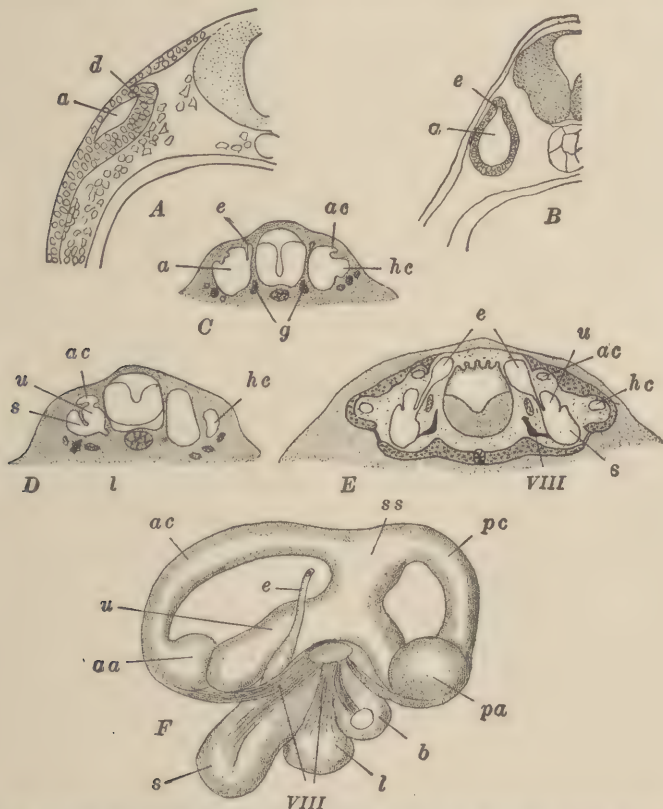
Fig. 98. — The development of the auditory organ in the Frog and Toad. From Kellicott (Chordate Development). A, B, F, after Krause; C, D, E, after Villy. A. Section through the auditory vesicle of an embryo just beginning to elongate. B. Section through the auditory vesicle that has very nearly separated from the superficial ectoderm. C. Transverse section, somewhat oblique, through the auditory organs of a 12 mm. *R. temporaria.* D. Slightly more advanced stage than C. E. Section through the auditory organs of a 25 mm. *R. temporaria.* F. Membraneous labyrinth of the Toad (*Bufo vulgaris*).

a. Auditory sac. *aa.* Anterior ampulla. *ac.* Anterior vertical semicircular canal. *b.* Pars basilaris. *d.* Dorsal outgrowth of primitive auditory vesicle (rudiment of endolymphatic duct). *e.* Endolymphatic duct. *g.* Ganglion of auditory (VIII) nerve. *hc.* Horizontal semicircular canal. *l.* Lagena or cochlea. *pa.* Posterior ampulla. *pc.* Posterior vertical semicircular canal. *s.* Saccule. *ss.* Sinus superior. *u.* Utricle. VIII. Auditory nerve.

its ends termed an *ampulla*. These ampullæ are not developed from the canals themselves, but are added to them through a further constricting off of portions of the utricle (Fig. 98, *E, F*).

Meanwhile the saccule in the course of its separation from the utricle has become the part of the otocyst which receives the endolymphatic duct. The two ducts, one from each side of the head, then grow up over the brain; during this process their ends become enlarged (at about 20 mm.) to form the *endolymphatic sacs*. By the time of metamorphosis, each sac has increased greatly in size, become very vascular, and fused with the corresponding sac. In the adult they form a considerable vascular covering for the myelencephalon.

In larvæ of 15–20 mm. the saccule is also giving rise to two other structures as follows: From its upper portion the *lagena* or *cochlea* arises as a postero-ventral evagination, while a similar outpushing, in close connection with the first, constitutes the *basilar chamber* (*pars basilaris*) (Fig. 98, *F*).

Sensory patches develop on the inside of the epithelial walls of the utricle, saccule, cochlea, and ampullæ, and these are connected with branches of the auditory nerve which proceeds from its ganglion. The entire membraneous labyrinth thus formed is eventually encased in cartilage and bone arising from the surrounding mesenchyme. The casing follows the contour of the membrane, and constitutes the *auditory capsule*. There is a slight space between the capsule and membrane, the *perilymphatic space,* and this is filled with *perilymphatic fluid*.

The Middle Ear. — This portion of the auditory organ develops chiefly during and after metamorphosis, as follows: The vestigial visceral pouch between the mandibular and hyoid arches; i.e., the hyomandibular, produces from its dorsal end a rod of cells with a terminal knob. This rod grows out until the knob reaches a position between the inner ear and the wall of the head. A cavity then develops in the knob and in the rod of cells. The cavity in the knob is the *tympanic cavity,* and that in the rod the *Eustachian tube,* which connects the cavity with the pharynx. The tympanic cavity, or cavity of the middle ear, increases in size until its outer wall fuses with the ectoderm. The membrane thus formed is the *tympanic membrane* or ear drum, separating the tympanic cavity from the exterior.

Upon its inner side, on the other hand, the wall of the tympanic cavity comes in contact with the auditory capsule. Here there is an
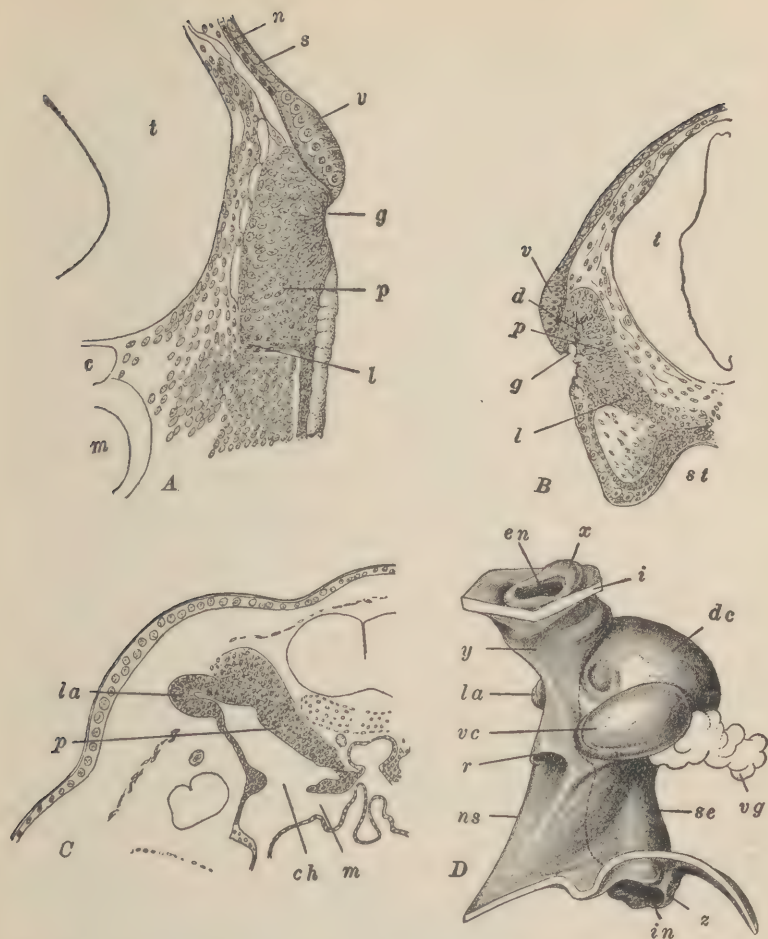
Fig. 99. — The development of the olfactory organ in *R. fusca*. From Kellicott (Chordate Development). After Hinsberg. A, B, C, Sections through the olfactory pit and organ of 5 mm., 6 mm., and 11 mm. larvæ, respectively. D. Lateral view of a model of the olfactory organ of a 31 mm. larva. The dotted line marks the limit between the sensory and non-sensory portions of the epithelial lining of the olfactory cavities.

c. Notochord. *ch.* Internal nares (choanæ). *d.* Dorsal lumen. *dc.* Dorsal sac. *en.* External nares. *g.* Olfactory pit. *i.* Cut edge of integument. *in.* Internal nares (choanæ). *l.* Elongation toward the mouth. *la.* Lateral appendix. *m.* Mouth cavity. *n.* Inner or nervous layer of ectoderm. *ns.* Part of chamber lined with non-sensory epithelium. *p.* Olfactory placode. *r.* Ridge marking the limit between middle and ventral chambers. *s.* Superficial layer of ectoderm. *se.* Part of the chamber lined with sensory epithelium. *st.* Stomodæum. *t.* Telencephalon. *v.* Thickened bands of superficial ectoderm cells (possibly the vestige of a primitive sense organ). *vc.* Ventral sac. *vg.* Ventral nasal gland attached to Jacobson's organ. *x.* Elevation around external nares. *y.* Canal leading to olfactory cavity. *z.* Fold around internal narial opening.

aperture in the latter, opening into the perilymphatic space, and called the *foramen ovale*. In the foramen ovale there develops a cartilaginous plug, the *operculum*. Across the roof of the tympanic cavity there is formed a cartilaginous rod, one end of which connects with the operculum and the other with the tympanic membrane. It is termed the *plectrum* or *columella*. Around the margin of the membrane there is forming meanwhile a third piece of cartilage, shaped like a ring, and known as the *annulus tympanicus*. It is said to arise as a small outgrowth from the posterior end of the *palato-quadrate* (see account of the skeleton). Finally at the close of metamorphosis the columella becomes separated from the dorsal wall of the tympanic cavity, so that it stretches freely across the cavity from the tympanic membrane upon the one hand to the operculum upon the other. The columella and operculum then become fused, and the latter and part of the former undergo gradually ossification.

There is no *outer ear*, the tympanic membranes appearing on the outside of the Frog's head.

The Olfactory Organ. — In the account of the external developments, we have already referred to the olfactory pits, which are evident, even in a 2.5 mm. larva. Each is situated slightly above and anterior to the side of the mouth. As these pits form, the superficial epithelium in this case disappears, while the inner invaginating layer thickens. These thickenings, which thus constitute the walls of the pits, may be termed the *olfactory placodes* (Fig. 99. Compare with Fig. 89 of the exterior for general location).

A little after hatching there grows inward and downward from the floor of each pit a solid rod of cells. These rods presently become connected with the pharynx just posterior to the stomodæum, and in tadpoles of 12 mm., each has acquired a lumen. Their openings into the pharynx thus form the *internal nares*.

Somewhat later the olfactory lobes develop from the cerebrum, as indicated above. From each of these lobes, cells are then proliferated, which mingle with other cells derived from the placodes. The two strings of tissue thus constituted seem to become the sheaths of the I or *olfactory nerves*. The actual fibers of these nerves, however, arise from neuroblasts in the placodes, and grow backward to the lobes.

Meanwhile the pits are enlarging as the *nasal cavities*, and the remainder of the placode cells line them as the nasal epithelium. In the course of growth the cavities are removed somewhat from the
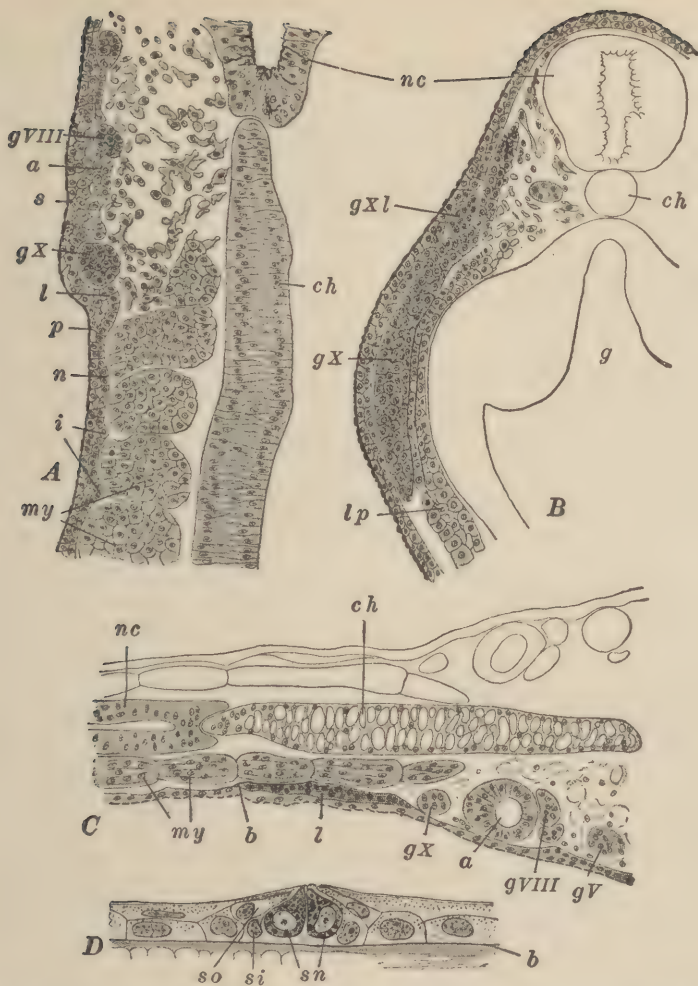
Fig. 100. — The development of the lateral line organs in *R. sylvatica*. From Kelli-cott (Chordate Development). After Harrison. A. Part of a frontal section through the level of the notochord of a 3.3 mm. embryo. B. Part of a transverse section through the vagus region of a 4 mm. embryo. C. Part of a frontal section through a 4 mm. embryo of *R. virescens*. D. Section through the lateral line organ of a 15.5 mm. larva of *R. sylvatica*.

a. Auditory vesicle (in A, its rudiment). *b.* Basement membrane of epidermis. *ch.* Notochord. *g.* Gut. *gV.* Trigeminal ganglion, of V cranial nerve. *gVIII.* Acoustic ganglion of VIII cranial nerve. *gX.* Vagus ganglion. *gXl.* Ganglion of the lateral line nerve (branch of the vagus). *i.* Intersegmental thickenings of the epidermis (ecto-derm). *l.* Rudiment of lateral line nerve. *lp.* Lateral plate of mesoderm. *my.* Myo-tomes. *n.* Inner or nervous layer of epidermis (ectoderm). *nc.* Nerve cord. *p.* Pig-ment in epidermis. *s.* Superficial layer of epidermis (ectoderm). *si.* Inner sheath cells of lateral line organ. *sn.* Sensory cells of lateral line organ. *so.* Outer sheath cells of lateral line organ.

surface of the head, but remain connected with it by tubes whose outer openings form the *external nares*. Changes in the shape and the proportion of the head alter from time to time the direction of the olfactory tracts. Thus these tracts first become vertical rather than horizontal, and later during metamorphosis develop a sharp flexure, due to the backward movement of the internal nares. At this latter period, also, each of the nasal cavities becomes greatly modified by complex evaginations and foldings. Of the former the most prominent arises ventro-medially from each cavity. The two bodies thus produced are the *organs of Jacobson;* they later acquire glandular masses at their medial ends.

The Lateral Line Organs. — At about 4 mm., a small dorso-lateral portion of the vagus ganglion of each side separates from the remainder and unites with a part of the most posterior or fourth placode. The placode then grows backward through the epidermis until, just before hatching, it reaches the tip of the tail (Fig. 100). At intervals along this cord there meanwhile arise groups of sensory cells which presently push their way to the surface and develop hair-like processes. These organs are innervated by a branch from the X nerve ganglion constituting the *ramus lateralis (lateral line nerve)*. Other similar sensory organs also develop in rows upon the head, and are innervated by branches of the VII, IX, and X nerves. All these organs disappear at metamorphosis.

INTERNAL DEVELOPMENT:
THE ALIMENTARY CANAL AND APPENDAGES

The Mouth

When last described, the endoderm in the antero-ventral part of the pharyngeal region of the fore-gut had pushed out an evagination toward the ectoderm. The ectoderm had also " pitted in " toward this evagination to form the stomodæum already noted. The stomodæal wall now meets and fuses with the endodermal wall in this region forming the *oral plate* or *oral membrane* (Fig. 90, *A*). A few days after hatching (about 9 mm.), the oral plate becomes perforated, and henceforth the stomodæal cavity or mouth communicates freely with the pharynx. The margins of the small larval mouth are formed fundamentally of

the *mandibular ridges;* i.e., the outer edges of the mandibular arches. Outside of these ridges, however, the skin is drawn forward to form the *dorsal* and *ventral lips.*

The dorsal lip of the larva soon develops three medially incomplete rows of "*teeth.*" Each of these teeth is formed from a connified ectodermal cell which is periodically replaced by a similar cell pushing up from beneath. The ventral lip has four rows of such teeth; these rows, however, are complete. At the base of each lip, parallel with the rows of teeth, is a hardened ridge or jaw, also formed of cornified ectoderm.

At metamorphosis the horny teeth and jaws are lost, the adult jaws being of course much wider than those of the larva and formed largely of elements derived from the mandibular arch (Marshall). The permanent teeth occur only on the upper jaw, and are similar in their general structure to mammalian teeth. The *tongue* develops at this time from a proliferation of cells in the floor of the pharynx.

The Fore-Gut and Its Derivatives

The Visceral Arches and Pouches. — The beginnings of the first three pairs of pouches arising as solid vertically elongated evaginations of endoderm have already been indicated. The most anterior pair are the rudiments of the hyomandibular pouches, whereas the second and third pairs are the rudiments of the first and second branchial pouches. There presently arise three more pairs of these solid rudiments, making in all six pairs, one hyomandibular and five branchial, the last pair, however, being mere vestiges. The condition of both pouches and arches at the time of hatching may now be summed up in the following manner (Fig. 101):

With the exception of the sixth and last, the pouch rudiments, as noted, push out until they finally reach and fuse with the ectoderm of the corresponding clefts. They thus divide the mesoderm into the following bars or visceral arches: (1) the mandibular arch in front of the first or hyomandibular pouch; (2) the hyoid arch between the hyomandibular pouch and the first branchial pouch; (3) the first branchial arch following the first branchial pouch; (4) the second branchial arch following the second branchial pouch; (5) the third branchial arch following the third branchial pouch; (6) the fourth

branchial arch, poorly defined, and following the fourth branchial pouch. There are thus six arches in all, beginning with the mandibular arch in front of the hyomandibular pouch, and ending with the fourth branchial arch in front of the last vestigial fifth branchial pouch.

The further development of the gill slits and gills has already been partially described in the account of the exterior. Nevertheless, it will be well at this point to recall the main features indicated, and to add certain details.

Fig. 101. — Diagram of a frontal section of a Frog larva at the time of hatching. From Kellicott (Chordate Development). After Marshall (modified). (" Vertebrate Embryology," courtesy of Putnam's Sons.)

c. Cœlom. d. Pronephric duct. F. Fore-brain. i. Infundibulum. in. Intestine. n. Nephrostome. o. Base of optic stalk. ol. Olfactory pit (placode). p. pharynx. t. Pronephric tubules. II. Hyoid arch. III–VI. First to fourth branchial arches. 1. Hyomandibular pouch. 2–6. First to fifth branchial pouches.

It will be remembered that, at about the time the mouth opens, the pharynx was said to be placed in communication with the exterior by means of the four pairs of branchial clefts and pouches. The changes in the solid pouches which make this possible, however, remain to be noted. Shortly after hatching, cavities appear in the first four pairs of branchial pouches, and these cavities become continuous with that of the pharynx. The cavities of the second and third pairs of branchial pouches then acquire openings to the outside by breaking through the points of fusion between the invaginated ectoderm and the endoderm, and the cavities of the first and fourth presently do likewise. The two hyomandibular pouches never develop any real cavities, however, and the tissue which composes them later disappears. Since, likewise, there are no cavities in the fifth vestigial branchial pouches, there are formed altogether but four pairs of actual gill slits.

It has been noted that after the external gills are covered by the

operculum they soon atrophy and are functionally replaced by the *internal gills*. On the first three pairs of branchial arches these consist of a double row of filaments situated just ventral to those which are disappearing, but upon the posterior side of each arch, rather than upon its outer face. There is also a single row of filaments upon the anterior side of each of the fourth branchial arches. It is due to the fact that these new gills are upon the sides of the arches instead of
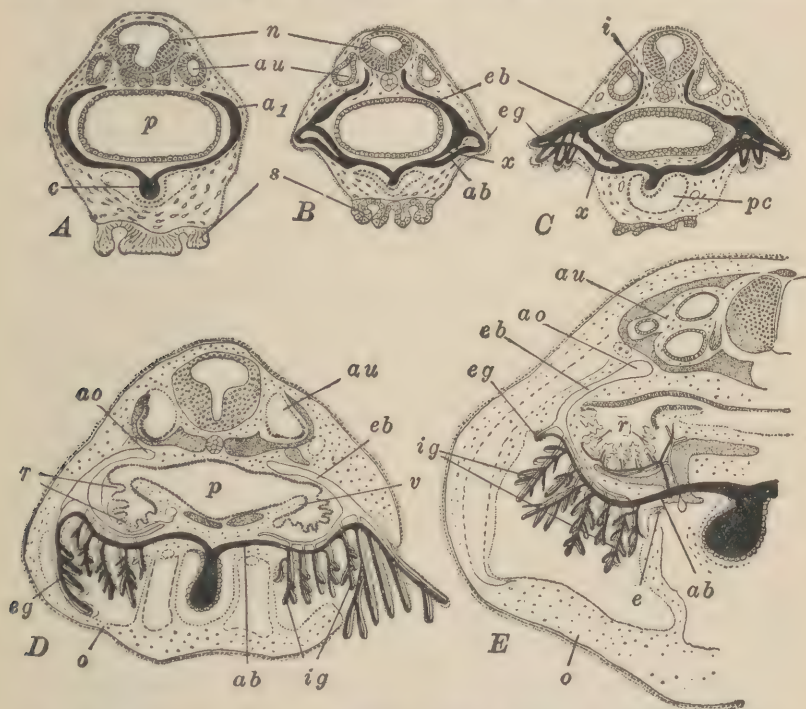
Fig. 102. — Sections through the branchial region of tadpoles of *R. esculenta*, showing the development of the gills and the history of the aortic arches. From Kellicott (Chordate Development). After Maurer. A. 4 mm. larva showing the continuous first branchial aortic arch. B. 5 mm. larva showing the anastomosis between the afferent and efferent portions of the aortic arch. C. 6 mm. larva with vascular loops in the external gills. D. 13 mm. larva. On the left the opercular chamber is closed and the external gill is beginning to atrophy, while on the right this chamber is still open and the external gill well developed and projecting through the opercular opening. E. 17 mm. larva. Vessels of the second branchial arch. External gill represented only by a minute pigmented vestige.

a_1. First branchial aortic arch. *ab*. Afferent branchial artery. *ao*. Root of lateral dorsal aorta. *au*. Auditory organ. *c*. Conus arteriosus. *e*. Epithelioid body. *eb*. Efferent branchial artery. *eg*. External gill. *i*. Internal (anterior) carotid artery. *ig*. Internal gills. *n*. Nerve cord. *o*. Operculum. *p*. Pharynx. *pc*. Pericardial cavity. *r*. Gill rakers. *s*. Oral " sucker." *v*. Velar plate. *x*. Anastomosis between afferent and efferent branchial arteries.

upon their outer faces that they are termed internal. Nevertheless, they are still ectodermal rather than endodermal, and project well into the branchial (opercular) chamber. Thus, save for the fact that they are covered by the operculum, the term internal as applied to them is something of a misnomer. Meanwhile during the development of these structures other changes have been taking place, as follows: First, owing to the inequalities in growth, there has been a considerable ventral shifting of the two branchial regions. Thus instead of being situated on the sides of the pharynx they soon come virtually to occupy its floor, upon either side of a relatively narrow median strip. For this reason the new gills do not project laterally, but tend to hang downward into the opercular chamber (Fig. 102). Just anterior and posterior to each of the two branchial regions there also develops from the pharyngeal wall; i.e., now the floor, a fold or flap termed a *velar plate*. These plates are so arranged, moreover, that upon each side, the anterior and posterior plates of that side almost meet one another, so that only a relatively narrow opening between them leads from the pharynx to the respective set of gill slits. It is these plates, together with toothlike processes on the inner sides of the gill arches, which tend to prevent the escape of food, while allowing the free passage of water. Finally at the time of metamorphosis the gill pouches and the gill cavity are filled by proliferated cells, while the mass thus formed is later absorbed leaving the gill slits closed.

Structures Derived from Vestiges of the Gill Pouches. — Just before hatching, proliferations of cells occur from the dorsal ends of the hyomandibular and first branchial pouches. Those from the hyomandibular pouch presently disappear, but those from each of the first branchial pouches form a cell mass. These separate from the pouches (about 12 mm.), and eventually take up their position back of the auditory capsules near the surface of the head. They are the *thymus bodies* (Figs. 103, 104).

From the ventral ends of the first pair of branchial pouches, cell proliferation occurs (9–10 mm.) which forms the *carotid glands*. From the ventral ends of the second and third branchial pouches the *epithelioid bodies* are similarly developed.

The fifth pair of branchial pouches never actually develop as such but become mere masses of tissue known as the *ultimobranchial bodies* (*suprapericardial*).

The Thyroid. — This organ appears before hatching as a median

longitudinal evagination from the floor of the pharynx in the form of a solid rod. Later (about 10 mm.), this separates entirely from the pharynx, and divides into two parts which eventually become vascular.

The Lungs. — They appear just after hatching as a pair of solid posteriorly directed proliferations from the ventral side of the pharynx just back of the rudiment of the heart. The pharynx at this point is later depressed, and partially constricted off
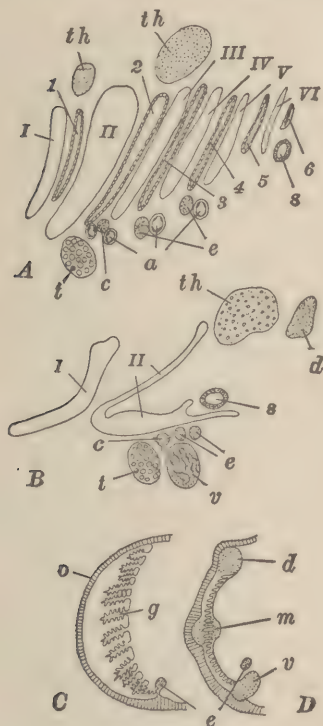
Fig. 103

Fig. 104

Fig. **103.** — Diagrams of the derivatives of the visceral pouches and arches in the Frog. From Kellicott (Chordate Development). After Maurer, with Greil's modification. A. Lateral view, Frog larva. B. Lateral view, after metamorphosis. C. Transverse section through gill of Frog larva. D. Transverse section through gill region just after metamorphosis; the gills have not quite disappeared.

a. Afferent branchial arteries. *c.* Carotid gland. *d.* Dorsal gill remainder. *e.* Epithelioid bodies. *g.* Internal gills. *m.* Middle gill remainder. *o.* Operculum. *s.* Suprapericardial or postbranch al body. *t.* Thyroid body. *th.* Thymus bodies. *v.* Ventral gill remainder. I–VI. visceral arches. I. Mandibular arch. II. Hyoid arch. III–VI. First to fourth branchial arches. 1–6. Visceral pouches (**1.** Hyomandibular pouch. **2–6.** First to fifth branchial pouches).

Fig. **104.** — Diagram of the branchial pouch derivatives in the Frog. From Kellicott (Chordate Development). After Maurer, with Greil's modification.

cg. Carotid gland. e_1, e_2, e_3. Epithelioid bodies. *th.* Thyroid body. tm_1, tm_2. Thymus bodies. *ub.* Ultimobranchial body. I–VI. First to sixth visceral pouches (I. Hyomandibular. II–VI. First to fifth branchial pouches).
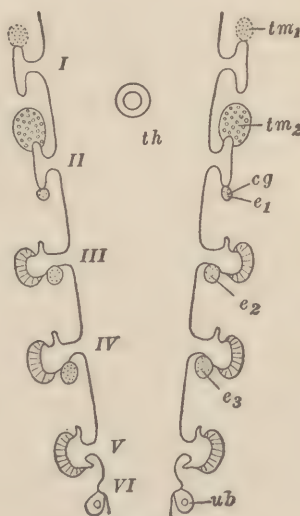
from the part above it as the *larynx*. The opening left between the pharnyx and larynx is the *glottis* (Fig. 90). The lungs soon acquire cavities, and as they grow, become spongy and vascular. Part of their tissue is derived from the splanchnic mesoderm, only the inner lining being endodermal.

Further Development of Liver. — The liver rudiment has already been noted as a small endodermal diverticulum extending back slightly, beneath the yolk mass. The anterior wall of this diverticulum becomes folded and thickened, partly by the addition of scattered mesoderm and yolk cells (Fig. 90). This is the liver proper, the posterior part of the original outgrowth becoming partially constricted away from it as the *gall bladder*. The original connection with the fore-gut remains as the *bile duct*. These organs become well developed during the larval stage.

The Pancreas. — At the posterior margin of the opening of the bile duct into the fore-gut, a pair of outgrowths arise connected with the gut by a single piece of tissue, the future *pancreatic duct*. The free ends of these outgrowths then grow forward and fuse in front of the bile duct. Later they are joined by a mass of tissue which originated from the dorsal wall of the gut, and the three elements thus fused constitute the *pancreas*. Eventually the pancreatic duct comes to open into the bile duct very near to the point where the latter joins the gut, instead of directly into the gut itself.

The Œsophagus and Stomach. — Shortly subsequent to hatching, the portion of the fore-gut between the future glottis and the opening of the bile duct elongates, and the anterior part of it becomes the *œsophagus*. For a brief time the aperture between the latter and the pharynx is closed, but reappears at about the time the mouth opens. The posterior part of the above fore-gut region dilates slightly and assumes a transverse position as the *stomach*. This organ remains inconspicuous, however, until the time of metamorphosis when it enlarges somewhat.

THE MID-GUT

The mid-gut is that portion of the archenteron lying above the large yolk mass at the time of hatching. After hatching, the yolk cells of its floor are rapidly absorbed, and it begins to elongate. The front portion extends across the body in the form of a loop, the *duodenum*, which with the remainder is soon thrown into a double spiral. The

coils of this spiral have a total length approximately nine times that of the body, but this is shortened about one-third during metamorphosis.

A peculiar and transitory structure developed in connection with this region is the *hypochordal rod*. It arises somewhat before hatching as a longitudinal string of cells constricted off from the dorsal wall of the mid-gut, between it and the notochord. Appearing first just back of the pancreas, it later extends even into the tail. It soon becomes separated from the gut by the development of the dorsal aorta, and shortly after hatching it disappears entirely.

THE HIND-GUT

The Rectum. — This terminal part of the gut originates with a relatively slight amount of growth from the small portion of the archenteron remaining between the yolk mass and the posterior body wall. It will be remembered that the endoderm of this region had come into contact with the ectoderm which had become invaginated to form the proctodæum. About a week before hatching a perforation occurs at the point of contact forming the *anus*, while the rectum itself becomes slightly dilated. In this connection it is of interest to note that the proctodæal portion of the blastopore which in the Frog closes with the rest of this orifice, and later reopens, in the Salamander always remains open. Thus the temporary closure in the former animal is probably a secondary or non-primitive characteristic.

The Postanal Gut. — As the tail region develops, the notochord and nerve cord extend into it, but since the proctodæal region does not move backward, the neurenteric canal is drawn out into a small tube beneath the posterior end of the notochord. Somewhat before hatching it breaks away from the neural tube and persists for a brief period as the *postanal gut*.

The Cloaca and Urinary Bladder. — The general region of the proctodæum later invaginates further so as to form a chamber, the *cloaca*, into which open the *urinogenital* ducts as well as the anus. Finally at metamorphosis the ventral part of the cloaca gives rise to an anteriorly directed outgrowth within the body cavity; this becomes the *urinary bladder*. It is to be noted in this connection that in the Frog the above ducts do not open into this bladder, but into the dorsal wall of the cloaca.

INTERNAL DEVELOPMENT:
THE FURTHER DEVELOPMENT OF THE MESODERM

THE SOMITES

When last considered, the segmental plates had divided into four pairs of somites. This process continues posteriorly until there are thirteen such pairs, extending from just back of the auditory capsules to the base of the tail. Within the latter organ the number is much larger and somewhat variable. Thus in a 5.5 mm. larva there may be all told as many as forty-five. Sometime after hatching, however, the first two pairs disappear, and those in the tail are of course all lost during metamorphosis; there thus remain eleven well-defined somites in the body region. Meanwhile, as these somites are formed they have been undergoing certain changes, as follows:

Each somite it will be recalled consists of an outer layer of cells called the cutis plate, and an inner larger mass, the myotome. From the inner and ventral edges of the myotomes (about 5 mm.), loose *sclerotomal cells* are proliferated (Fig. 87). These cells then migrate medially and dorsally between the rows of myotomes on the one hand, and the notochord and nerve cord on the other. Eventually they thus form a layer about the latter structures known as the *skeletogenous sheath*. This ultimately (see below) gives rise to the cartilage and finally the bone which forms the centra of the vertebræ together with their transverse processes and neural arches. There are nine vertebræ thus formed in such a way that they alternate with the myotomal elements of the somites. The skeletogenous elements of the last two of the eleven somites have a somewhat different history, as indicated later in the account of the vertebral column.

At about the same time that the sclerotomal tissue is being proliferated, there are developing, within the myotomes, *muscle fibrillæ*, which are to form the muscles of the back. Also from the outer ventral edges of the myotomes and from the ventral edges of the cutis plates or dermatomes, outgrowths extend down next to the ectodermal wall. These are to form the ventral body musculature, and in the region of the limbs, their musculature as well. The main part of each cutis plate breaks up and some of the cells from these plates form the dermal layer of the dorsal region, while others migrate be-

tween the myotomes to form connective tissue. Finally, as indicated above, the mesoderm in the region where the segmental plate separates from the lateral plate constitutes the nephrotome, and is concerned with the formation of the execretory system. This will be described later.

THE GENERAL CŒLOM

The beginning of the cœlomic spaces in the two lateral plates has already been described. These spaces continue to extend downward, until in a short time they meet one another beneath the gut and fuse. Thus in the trunk region, the cœlom or *splanchnocœl* becomes continuous ventrally from one side of the embryo to the other.

Dorsally, the lateral plates of mesoderm on each side press up and in, between the dorsal wall of the gut and the notochord, until they meet. The splits in these plates then follow, but never quite reach each other, and hence the splanchnocœl never becomes continuous dorsally; there is always a thin but double-walled sheet of cells separating the right and left cavities. This is the *dorsal mesentery*. The gut as it develops is therefore slung from the dorsal wall by this mesentery, and completely encased in the splanchnic mesoderm.

INTERNAL DEVELOPMENT:
THE CIRCULATORY SYSTEM

THE HEART AND PERICARDIAL CAVITY

The Primitive Cardiac Tube. — It will be recalled that when last mentioned the heart consisted merely of a few scattered endothelial cells lying between the endodermal floor of the pharynx and the mesoderm. It will also be remembered that upon either side of the mid-line this mesoderm had developed within itself a space which was designated as a rudiment of the pericardial cavity (Fig. 88, *C*). These spaces now enlarge, and the mesoderm forming their uppermost walls presses up and around each side of the above mentioned endothelial cells so as to separate them from the overlying pharynx. Meantime these cells have become arranged in the form of a tube which extends throughout the region. Presently the in-pushing mesoderm from either

side meets and fuses above the tube, so as entirely to surround it (3–6 mm.), (Fig. 105; cf. also, Fig. 88, *D*). The latter with its covering now represents the complete rudiment of the *heart*. The endothelial portion, as noted, forms its lining, the *endocardium*, while the mesodermal envelope gives rise to the muscular wall, or *myocardium*.

From the method of its formation, it is evident that this tubular heart will at first be attached to the walls of its pericardial cavity by both a dorsal and ventral sheet of mesodermal epithelium, or *mesocardium*. The dorsal sheet was formed like that which suspends the gut, by the fusion of the sheets of mesoderm pushing in from each side. The
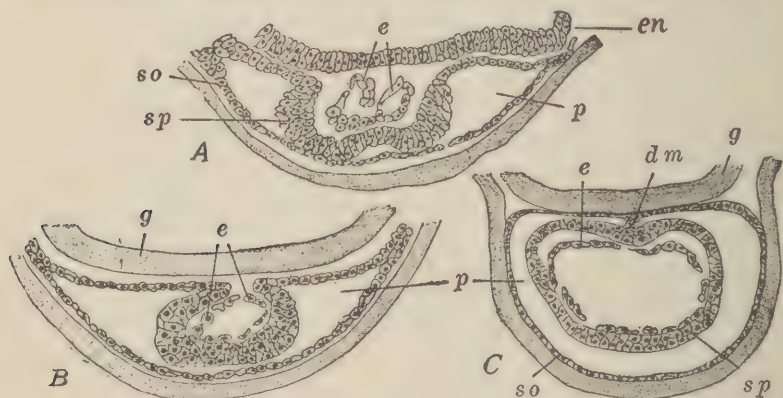
Fig. 105. — Sections showing the formation of the heart in the Frog.   From Kellicott (Chordate Development).   A. Section through pharyngeal region of *R. temporaria*. After Brachet.   B, C. Sections through the same region in older embryos of the smaller Frog, *R. sylvatica*.   A. 3.2 mm. embryo.   Endothelial cells becoming arranged in the form of a tube.   B. Embryo of about 3 mm.   C. Embryo of 5–6 mm.   Heart tube established; dorsal mesocardium still present.

dm. Dorsal mesocardium. *e.* Cardiac endothelial cells. *en.* Endoderm. *g.* Wall of gut (pharynx). *p.* Pericardial cavity. *so.* Somatic layer of mesoderm (here, wall of pericardial cavity). *sp.* Splanchnic layer of mesoderm (here, myocardium).

ventral sheet, on the other hand, has existed from the start as the median strip separating the two pericardial rudiments. Thus the pericardial space remains temporarily divided along this middle line. Meantime, as indicated above, the lateral cœlomic spaces in the trunk region have extended ventrally, and now each side of the pericardial cavity communicates posteriorly with these spaces. The next step involves the entire disappearance of the ventral mesocardium, followed very soon by the disappearance of the dorsal mesocardium also, except at its anterior and posterior ends.

As the mesocardia disappear, the tubular heart begins to increase in length, and by so doing becomes folded in the form of a letter S. If now the animal be regarded as lying upon its right side with its head toward the reader's left, the left side of the letter as it appears on the page will represent the anterior side of the heart and the right side of the letter the posterior side of the heart. With respect to the position of the animal, the top of the letter might then be thought of as exactly dorsal and the bottom ventral. As a matter of fact, however, the relation is not quite this because the position of the heart at first is slightly oblique. Thus the top of the letter must be thought of as tipped somewhat toward the reader and the bottom away from him. Presently, however, as a result of the ventral movement of the lower portion of the figure (i.e., in the direction of the reader), it becomes approximately vertical. The future regions of the heart may now be oriented with respect to the parts of the letter as follows: The upper (dorsal) portion represents the rudiment of the *auricles;* the middle and part of the lower portion represent the future *ventricle;* and the remainder of the lower (ventral) portion, the *bulbus aortæ.* The more detailed development of these parts may now be described as follows:

The Auricular Region. — The upper or auricular region is evidently the posterior end of the previously straight tube, and still abuts against the liver (Fig. 90, *A*). Meantime, two veins passing forward along the sides of that organ have come to open into this auricular portion. They are the *vitelline veins,* and at the point of entrance to the auricle they become fused; the fused region is then termed the *sinus venosus* (Fig. 90, *B, sv*). Still later there grows down from the roof of the auricular chamber within the heart a longitudinal sheet of tissue, which is termed the *interauricular septum,* and which divides it into right and left halves. This division, moreover, occurs in such a manner that the sinus venosus comes to empty into the right auricle, while the left auricle eventually receives the pulmonary veins (see below).

The Ventricular Region. — The middle or ventricular portion of the S is largely undivided in the Frog. Its walls, however, soon become greatly thickened by the development of muscular tissue, fibers of which traverse the ventricle itself. Finally the most ventral portion of the letter forms, as noted, the bulbus aortæ, from the anterior end of which two strands of cells grow forward beneath the pharynx.

These soon fuse, and develop a common lumen, which becomes divided by a longitudinal septum into right and left channels. It is the *truncus arteriosus* (Fig. 90, *B, ta*).

Isolation of the Pericardial Cavity. — Most of the above processes take place in the development of the heart before or shortly after the tadpole hatches (7–12 mm.). One step which remains until considerably later, however, is the separation of the pericardial cavity from the general cœlom which lies posterior to it. This is accomplished by the outgrowth of folds of peritoneum (epithelial lining of the cœlom) from the lateral cœlomic walls, in company with the ductus Cuvieri (see below). The partial transverse wall thus formed is then augmented medially by the splitting off of peritoneal tissue from the anterior face of the liver. The entire partition is not completed until metamorphosis, when it is known as the *septum transversum*.

Development of Blood Vessels and Corpuscles

The blood vessels develop out of the mesenchyme and the splanchnic mesoderm by a rearrangement of the cells. The corpuscles are formed chiefly from patches of splanchnic mesoderm on the ventral side of the yolk mass, from whence they find their way into the developing vessels. These patches are called *blood islands*.

The Arterial System. — A few days before hatching (4–5 mm.), the *dorsal aorta* develops in the manner indicated, just above the gut, and in the pharyngeal region is divided into two *lateral dorsal* or *suprabranchial aortæ*.

The Visceral Arch and Gill Circulation. — At about the same time the blood vessels of the visceral arches also develop in the following manner:

Vessels appear in the branchial arches and a little later the two anterior pairs become connected ventrally with the truncus arteriosus and dorsally with the corresponding suprabranchial aorta. Presently similar connections are also established by the other two pairs. Thus complete loops or *aortic arches* are formed in all but the mandibular and hyoid arches. Here no real aortic arches ever develop, though certain transitory vessels appear for a time.

As the external gills now begin to form, the following changes occur in the first, second, and third branchial arches: A second looped

vessel appears external to the primary aortic (branchial) vessel, the new vessel being attached to the primary vessel dorsally and ventrally (Figs. 102, *C;* 106, *B*). This new loop now extends out into the tissue of the corresponding external gill, where the two sides of the loop are connected by capillaries. Thus, for the time being, it is possible for the blood to go through either the original primary loop or the new gill loop and its capillaries. The greater part of the blood, however, takes the latter course. Hence it passes out from the truncus arteriosus along the more ventral side of the gill loop, which is therefore *afferent,* and back along the dorsal side, which is therefore *efferent.*

When the external gills disappear, the more proximal portion of the ventral limb of the external loop remains to form the afferent vessel
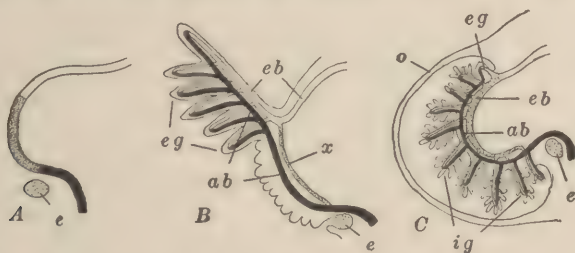
Fig. 106. — Diagrams of the second aortic arch of the adult Frog and tadpole. From Kellicott (Chordate Development). After Maurer. A. The continuous second (main systemic) aortic arch of the adult; showing the parts corresponding with the larval vessels. B. External gill and associated vessels in young tadpole. C. Internal gill and associated vessels in the tadpole after the disappearance of the external gills.

ab. Afferent branchial artery. *e.* Epithelioid body. *eb.* Efferent branchial artery. *eg.* External gill. *ig.* Internal gill. *o.* Operculum. *x.* Direct anastomosis between afferent and efferent branchial arteries; i.e., ventral part of primary loop.

of the internal gills (Figs. 102, 106). The efferent vessel, with which it then becomes connected by capillaries, is the more ventral part of the original primary loop. Meanwhile, this primary loop breaks its main ventral connection at the point where the external loop branched off from it. Thus during the remainder of larval life all the blood in the arches has to go through the internal gill capillaries. Since the fourth arch never develops external gills, the vessels related to these particular structures never appear in it. Otherwise the history of the blood system within this arch is essentially similar to that just described in those anterior to it.

Changes in Gill Circulation at Metamorphosis. — As the gills and their capillaries are degenerating, the original primary loop vessels

reëstablish their ventral connections with the proximal parts of the afferent gill vessels. The primary vessels in the four pairs of branchial arches then undergo the following changes.[2] The vessels of the first
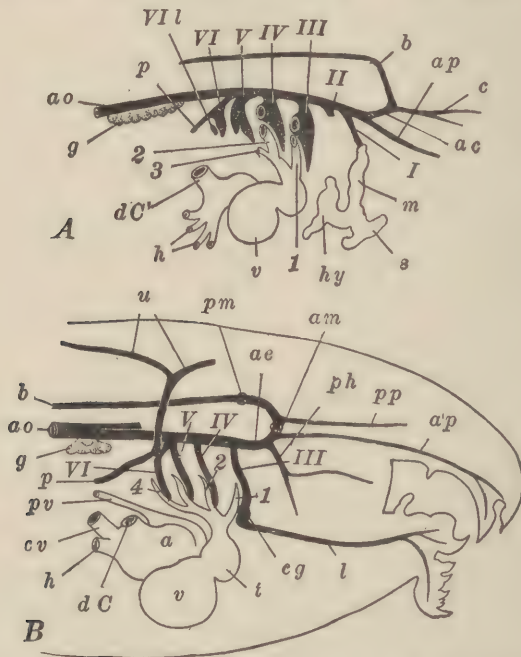
Fig. **107.** — Diagrams of the branchial blood vessels in Frog larvæ. From Kellicott (Chordate Development). After Marshall. ("Vertebrate Embryology," courtesy of Putnam's Sons.) A. Of a **7** mm. larva (shortly after hatching). The vessels supplying the external gills are removed, only their roots being indicated. B. Of a **12** mm. tadpole. The vascular loops in the gills are omitted.

a. Auricle. *ac.* Anterior (internal) carotid artery. *am.* Anterior commissural artery. *ao.* Dorsal aorta. *ap.* Anterior palatine artery. *b.* Basilar artery. *c.* Anterior cerebral artery. *cg.* Carotid gland. *cv.* Posterior (inferior) vena cava. *dC.* Ductus Cuvieri. *g.* Pronephric glomus. *h.* Hepatic veins. *hy.* Hyoidean vein. *l.* Lingual artery. *m.* Mandibular vein. *p.* Pulmonary artery. *ph.* Pharyngeal artery. *pm.* Origin of posterior commissural artery. *pp.* Posterior palatine artery. *pv.* Pulmonary vein. *s.* Vein of oral sucker. *t.* Truncus arteriosus. *u.* Cutaneous artery. *v.* Ventricle. **1–4.** First to fourth afferent branchial arteries. I, II. Efferent arteries of the mandibular and hyoid arches. III–VI. First to fourth efferent branchial arteries. VI *l.* Lacunar vessel of the fourth branchial arch.

[2] It is to be noted in this connection that at least in some Frogs, as indicated in a preceding paragraph, no genuine aortic loops are formed in the mandibular and hyoid arches (Marshall and Bles on *R. temporaria*). In many other Vertebrates or their embryos, however (see the Chick), complete arteries do exist in these arches at one time or another, as well as in the four branchial arches. Thus in such cases the third aortic loop of the entire series is homologous with that in the first branchial arch referred to in the following account.

pair of branchial arches retain their dorsal connections with the respective dorsal aortæ, and with them form the proximal ends of the *internal carotids* which run forward into the head (Fig. 107). The vessels of the same arches are joined at their bases by the *external carotids* or *lingual arteries* which have grown back from the floor of the mouth. The carotid glands (see above) become located at the point of junction of the external and internal arteries upon either side. The
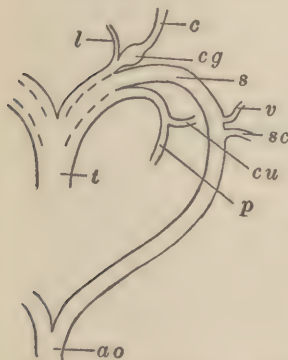
Fig. 108. — Diagram of the aortic arches and their chief branches in an adult Frog. From Kellicott (Chordate Development). Ventral view.

ao. Dorsal aorta. *c.* Carotid artery. *cg.* Carotid gland. *cu.* Cutaneous artery. *l.* Lingual artery. *p.* Pulmonary artery. *s.* Systemic arch. *sc.* Subclavian artery. *t.* Truncus arteriosus. *v.* Vertebral artery.

vessels of the second pair of branchial arches also retain their dorsal connections with the lateral dorsal aortæ, while the latter disappear anteriorly between this point and the first branchial arches (disappearance not shown in Fig. 107). Thus the vessels of the second branchial arches become the *main systemic arteries.* The vessels of the third branchial arches disappear. The vessels of the fourth branchial arches, having already given off branches to the lungs and skin, become the *pulmocutaneous arteries.* The portion of each of these vessels connecting it with the respective lateral aorta disappears after metamorphosis, except for a vestigial strand, the *ductus Botalli.* Thus all the blood going to these aortic arches must henceforth pass to the lungs or skin.

In conclusion it must be added that there appear in the truncus arteriosus at this time, septa which divide it into three channels (Fig. 108). One of these probably receives completely aerated blood from the left side of the heart, and guides it into the carotids. A second channel connects less directly with the left part of the heart, and apparently receives mixed blood, which it carries into the main systemic arteries. The third comes from the right of the heart, and carries unaerated blood to the pulmocutaneous vessels.

Other Arteries. — The *pharyngeal arteries* develop at about 9 mm. from outgrowths of the suprabranchial aortæ, which at first connect with transitory vessels in the mandibular arches. At about the middle of each main systemic aortic arch a large branch is given off to the

fore limb; it is the *subclavian*. The suprabranchial or lateral aortæ come together to form the single dorsal aorta at about the level of the pronephros (see below). Throughout the remainder of its course this artery gives off several *lumbar arteries* to the body wall, as well as larger branches which supply the viscera (*mesenteric arteries*), and the hind limbs and adjacent regions (*iliac arteries*).

The Venous System.

The Hepatic and the Hepatic Portal Systems. — In discussing the development of the heart it was noted that almost from the first two veins entered it posteriorly; i.e., the vitelline veins. Just at the point of entrance to the heart their fusion resulted in the formation of a common chamber, the sinus venosus. Between this point and the liver a further fusion of these veins occurs not long after hatching, and the result is called the *hepatic vein* (Fig. 107). Although first mentioned in connection with the heart, the vitelline veins actually appear first on the ventro-lateral sides of the yolk mass, whence they pass along the side of the yolk and liver to the heart. As noted, fusion early occurs anterior to the liver, but posterior to it the vitelline veins remain separate. The right vein within this region then disappears, and the left becomes the *hepatic portal vein*. It remains connected with the anterior hepatic vessel only through capillaries within the liver substance, while posteriorly it sends branches to the digestive tract. This vein with its branches and liver capillaries constitutes the *hepatic portal system.*

The Cardinal Veins and Their Derivatives. — Very early (before hatching) there appears upon each side of the animal, a vessel which runs from the sinus venosus obliquely in a dorso-lateral direction to the body wall; these vessels are the *ductus Cuvieri*. In the wall each sends a branch anteriorly and posteriorly. The proximal portion of the anterior branch is the *anterior cardinal vein*, while the more anterior or distal portion of the same vessel is called the *superior jugular vein*. Near the base of each ductus Cuvieri another vein is given off anteriorly. These veins bring blood from the ventral part of the head and the mouth, and are called the *inferior jugulars*.

As indicated, each ductus Cuvieri also gives off a posterior branch, the *posterior cardinal vein*. Each of these veins passes through the pronephric region in the form of a sinus which more or less surrounds the pronephric tubules (see below). Posterior to this region, it then continues along the median side of the respective pronephric (Wolffian)

duct to the cloaca. Along its course, each of the cardinals receives branches from the body wall, and at their posterior extremities the two veins unite and receive the *caudal vein* which brings the blood from the tail.

As the mouth opens and the mesonephros (permanent kidney) develops, these posterior cardinal veins are modified, as follows: Their posterior parts fuse to form a *median cardinal* vein (Fig. 109), while on the dorsal surface of the liver a new vein forms and becomes con-
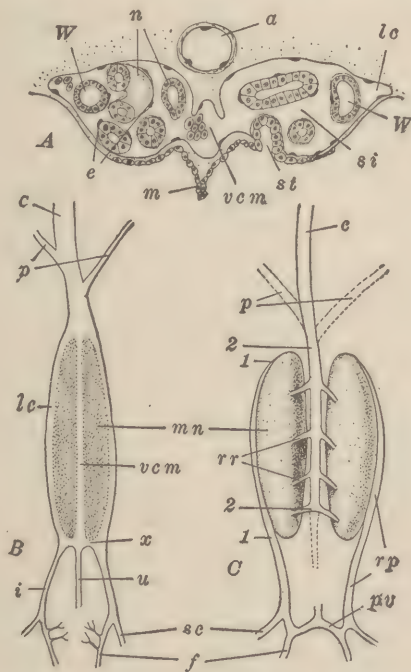
Fig. 109. — The development of the posterior part of the venous system in the Frog From Kellicott (Chordate Development). After Shore. A. Portion of a transverse section through the posterior mesonephric region of an 18 mm. tadpole. B. Diagram of the veins of a 25–30 mm. tadpole. C. Diagram of the veins of the adult Frog.

a. Dorsal aorta. *c*. Vena cava. *e*. Nuclei of the endothelial lining of the mesonephric sinus, continuous with the vascular endothelium. *f*. Femoral vein. *i*. Iliac vein. *lc*. Lateral mesonephric channel of the posterior cardinal vein. *m*. Mesentery. *mn*. Mesonephros. *n*. Mesonephric tubules. *p*. Posterior cardinal veins (in C showing their original location). *pv*. Pelvic vein. *rp*. Renal-portal vein. *rr*. Revehent renal veins. *sc*. Sciatic vein. *st*. Nephrostome. *u*. Caudal vein. *vcm*. Median mesonephric channel of the posterior cardinal vein. *W*. Wolffian duct. *x*. Connection between caudal vein and the lateral mesonephric channels. 1–1. Part of the renal-portal vein formed from the lateral channel of the posterior cardinal. 2–2. The posterior part of the vena cava formed from the median channel of the posterior cardinal vein.

nected anteriorly with the hepatic vein. Posteriorly it joins the right posterior cardinal vein just back of the pronephros and anterior to the point of fusion of the cardinals. The right cardinal, anterior to the point where the new vein has joined it, and all of the left cardinal now disappear. The single median vein which results is called the *posterior vena cava*. It is to be noted that its posterior portion is really simply the former median cardinal vein. This change leaves each ductus Cuvieri connected at its roots with an inferior jugular, and at its dorsal end with an anterior cardinal only. The portion of each ductus between these points, constituting the main part of the vessel, is then often referred to as an *anterior vena cava*. Presently the posterior vena cava increases in size so that the hepatic vein opens into it, rather than the reverse relation. Thus this caval vein may now be said to open directly into the sinus venosus.

As the pronephros disappears, the mesonephros develops, and in the region of the latter, the median cardinal, or as it is now called, the posterior vena cava, becomes divided longitudinally into three parts (Fig. 109, *B, C*). The median part continues as the permanent posterior portion of the posterior vena cava, whose caudal connection disappears with the lost of the tail. It receives blood from the kidneys through short *renal veins*. The lateral parts of the original caval vein, on the other hand, come finally to lie along the outside edge of each mesonephros (kidney), into whose capillaries their blood empties. Meanwhile each has been joined by an *iliac vein* coming from the respective hind leg, and a *dorso-lumbar vein* from the body wall. Thus these lateral portions of the caval vein, together with the iliac and dorso-lumbar veins constitute the *renal portal system*.

The Pulmonary Veins. These begin to develop very early (6 mm.) as a dorsal offshoot from the sinus venosus. Later this opens into the left auricle, while at the lungs it divides so as to receive blood from each.

The Abdominal Vein. At a relatively late period, a pair of veins from the sinus venosus extends back along the ventral body wall to the bladder, making lateral connections with the iliac veins. Just anterior to the bladder the two vessels then fuse, while still further forward the right one later disappears entirely. The remaining single vessel is the *abdominal vein*, which finally loses its connection with the sinus venosus and opens into the hepatic portal vein.

The Lymphatic System. — Just before hatching, the *anterior*

lymph hearts appear as hollow outgrowths from a pair of veins which usually pass between the fourth and fifth myotomes. They lie between the peritoneum and the integument, and soon become incased in muscle fibers. Each " heart " sends out a vessel anteriorly and posteriorly just beneath the skin, and these give rise to numerous anastomosing capillaries; the latter eventually form the characteristic *subcutaneous lymph sacs* of the Frog. Sometime after hatching (26 mm.), the

Fig. 110. — Ventral, lateral and dorsal views of the lymphatics in a **26 mm.** tadpole of *R. temporaria*. From Hoyer. For description see text.

anterior vessels open downwards into large lymph sinuses in the branchial region (Fig. 110). The lateral posterior trunks unite at the root of the tail, and then divide into a dorsal and a ventral vessel, which pass into it. The *thoracic ducts* appear to be outgrowths of the anterior lymph hearts, which extend posteriorly between the dorsal aorta and the posterior cardinal veins. Upon the appearance of the hind legs *posterior lymph hearts* develop from the segmental veins of that region also.

All the lymph hearts are guarded by valves between themselves

and the lymph channels on the one hand, and between the hearts and blood vessels on the other. Thus the lymph always passes into the blood, never in the reverse direction.

The Spleen. — At about 10 mm. there appears in the mesentery, on the mesenteric artery, just dorsal and posterior to the stomach, a collection of lymph cells. They multiply, and later (25 mm.) the cell mass becomes very vascular. The body thus formed is the *spleen.*

INTERNAL DEVELOPMENT:

THE LARVAL EXCRETORY SYSTEM

Although both the larval and adult systems are paired, we shall refer only to the development upon one side. This is done with the understanding that the processes on the opposite side are identical.

THE PRONEPHROS OR HEAD KIDNEY, AND THE SEGMENTAL DUCT

The Pronephros. — When last described, the somatic wall of the nephrotomal region had thickened until it slightly overhung the side of the lateral plate between it and the ectoderm; in the region of the second, third and fourth somites, cavities were beginning to appear within the thickening, especially in its outer portion (Fig. 86). These outermost cavities now tend to run together so as to form in this region a continuous longitudinal lumen, the *common trunk*. At the same time, other spaces between this lumen and the cœlomic cavity enlarge and unite with one another to form three separate tubules connecting the trunk with the cœlom. These are the *pronephric tubules*, and each of them is opposite one of the three somites referred to. The opening of each tubule into the cœlom is in the form of a funnel named the *nephrostome* (Fig. 111), which presently becomes lined with long cilia. The tubules, together with the common trunk, now become somewhat convoluted, and these convolutions begin to become imbedded in the sinus like cardinal vein which partially surrounds them (Figs. 111, 112). At the same time the mass which is thus formed becomes enclosed on its dorsal and outer sides by connective tissue derived from the myotomes of this region and from the somatic mesoderm. This covering is termed the *pronephric capsule*.

Although not directly connected with the pronephric tubules, there develops with them another organ which because of its position and structure is probably concerned with their function. It arises as an outpushing or fold of splanchnic mesoderm at the extreme dorsal
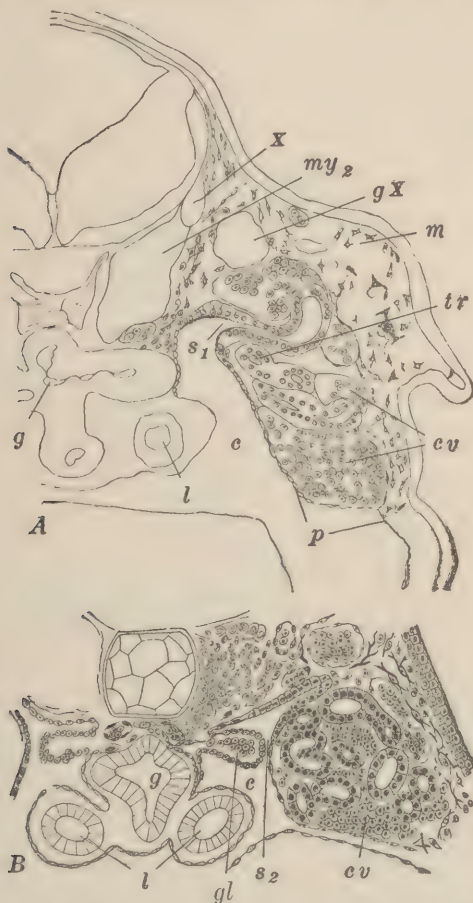
Fig. 111. — Sections through Frog larvæ illustrating the later development of the pronephros. From Kellicott (Chordate Development). A. Through the first nephrostome of a larva of *R. sylvatica* of about 8 mm., with prominent external gills. ; After Field. B. Through the region of the second nephrostome of a 12 mm. larva of *R. temporaria*. After Fürbringer.

c. Cœlom. *cv.* Sinuses of posterior cardinal vein. *g.* Gut cavity. *gl.* Glomus. *gX.* Ganglion nodosum (part of the ganglion of the vagus nerve). *l.* Lung. *m.* Mesenchyme. *my₂.* Second myotome. *p.* Peritoneum. *s₁, s₂.* First and second pronephric nephrostomes. *tr.* Common trunk. *X.* Root of vagus nerve.

limit of the cœlom in the region just opposite the nephrostomes. In this way a small mass of tissue becomes suspended directly opposite these openings. Presently numerous capillaries form within it and become connected with the nearby dorsal aorta. This vascular body is then called the *glomus*. The pronephric tubules, together with the glomus, may henceforth be referred to as the *pronephros* or *head kidney* (Figs. 112, 113).

The Segmental Duct. — So far as has yet been indicated, the larval kidney has no external outlet. While the above changes are
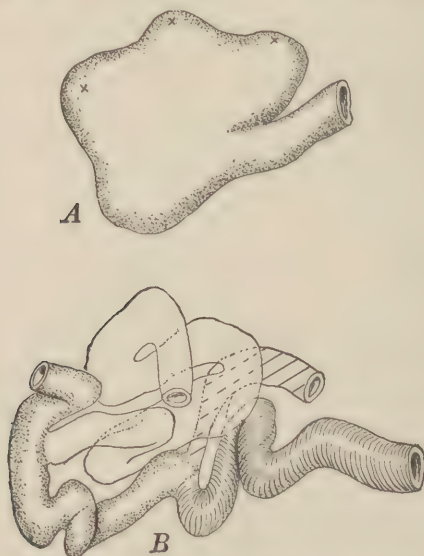
Fig. 112. — Total views of the pronephros of the Frog (*R. sylvatica*). From Kellicott (Chordate Development). After Field. A. Right pronephros of an embryo of about 3.5 mm. The crosses mark the location of the nephrostomes. B. Right pronephros of a larva of about 6 mm. First tubule dotted; second white; third obliquely ruled; pronephric (segmental) duct shaded with lines.

going on, however, the lumen of the common trunk has extended backward through the lateral border of the nephrotome until it has established a connection with the cloaca. The outer portion of the nephrostome containing this lumen is then called the *pronephric* or *segmental* duct. Posterior to the fourth somite it gradually becomes more or less separated from the more median portion of the undifferentiated nephrotomal tissue which occurs in this region.

Changes Subsequent to Hatching. — This is approximately the con-

dition reached at the time of hatching, when the tadpole is from 6–7 mm. long. The pronephros does not attain its maximum development, however, until the animal is about 12 mm. in length. During this period the pronephric tubules increase their convolutions to a considerable extent, and the cœlomic space into which the nephrostomes open and in which the glomus is suspended becomes cut off ventrally from the main cœlomic cavity. This is accomplished by the development of the lungs in this region (Fig. 111). These organs are covered
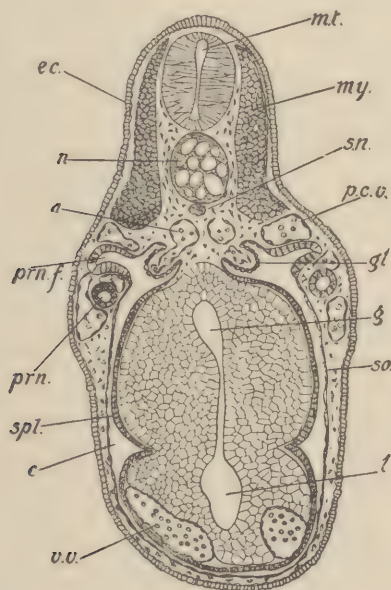
Fig. 113. — Transverse section of an advanced Frog embryo. From Jenkinson (Vertebrate Embryology).

m.t. Medullary tube. *n.* Notochord. *s.n.* Subnotochordal rod. *my.* myotome. *a.* Aorta. *p.c.v.* Posterior cardinal vein. *prn.* Pronephric tubule. *prn.f.* Pronephric funnel (i.e. nephrostome). *gl.* Glomus. *c.* Cœlom. *so.* Somatopleure. *spl.* Splanchnopleure. *g.* Gut. *l.* Liver. *v.v.* Vitelline vein. *ec.* Ectoderm.

by a fold of the splanchnic mesoderm, and, as they grow, this covering is finally brought into contact with the somatic mesoderm, with which it fuses for a short distance. The cavity thus formed, though separated from the cœlom beneath, remains open to it both anteriorly and posteriorly. It is termed the *pronephric chamber*.

By the time the larva reaches a length of 20 mm., the head kidney begins to degenerate. Thus the pronephric region of the segmental

duct becomes cut off from the part posterior to it. The former portion of the duct, together with the pronephric tubules and their nephrostomes, then gradually disappears;[3] the glomus at the same time shrivels up, though remnants are visible even after metamorphosis. As the larval kidney is thus eliminated, its place is taken functionally by the mesonephros whose development is now to be described.

THE MESONEPHRIC OR WOLFFIAN BODY

Posterior to the pronephros the outer margin of the nephrotome went to form the segmental duct. The inner portion, between the duct and the dorsal aorta in the region of the posterior cardinal vein, now starts to form the adult kidney.

The Mesonephric Vesicle. — As indicated above, this inner portion is for a brief time divided into segmental nephrotomes. These, however, disappear almost at once so that a single nephrotomal band extends from the seventh to the twelfth somites. Within this band there then arise a series of thickenings somewhat more numerous than the somites, and in each thickening there soon appears a cavity (Figs. 114, 115). This cavity, which is called the *mesonephric vesicle,* eventually becomes divided into two parts, the second and smaller part still later giving rise to a third. These parts are called *primary, secondary, and tertiary units,* in the order of their appearance, and their further development, though not simultaneous, is identical in character. It will be necessary, therefore, to describe the process in only one of the *primary* units.

The Development of a Primary Vesicular Unit. — Upon the dorsal side of the unit a small hollow outgrowth appears (Fig. 115, *B*). This, as later events prove, represents the rudiment of the secondary unit, but for the present does not develop further. Next (Fig. 115, *C*), an evagination pushes out from the ventro-lateral side of the primary unit in the direction of the segmental duct. This is the *inner tubule,* which presently becomes connected with the segmental duct, the latter being henceforth known as the *mesonephric* or *Wolffian duct.* It is to be noted, moreover, that, by virtue of the partial rotation of the primary unit, this connection occurs dorsally rather than ventrally (Fig. 115, *D, E*). A part of the inner tubule later becomes greatly convoluted and the coils press up into the median cardinal vein

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[3] Hall states that during the degeneration of the pronephros the three nephrostomal openings, at least in *R. sylvatica,* always become fused into one, the *common nephrostome* (Fig. 114, *C*).

(15 mm.), helping to divide the latter, as indicated above. Meanwhile there has grown out from what is now the ventral side of the unit, another evagination which presently becomes connected with the peritoneal (cœlomic) cavity. This is the *outer tubule*, whose subsequent history in the Frog is very peculiar.[4] It soon (20 mm.) breaks away from the main portion of the unit and acquires an opening into the lateral division of the median cardinal vein; i.e., the future renal portal vein. At the same time its opening into the cœlomic cavity becomes
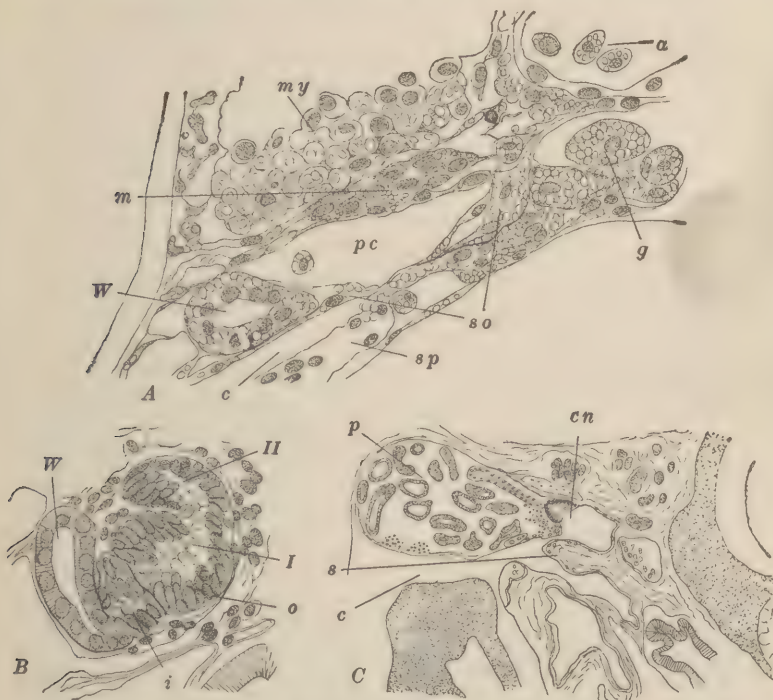
Fig. 114. — Sections through the developing mesonephros and the degenerating pronephros of *R. sylvatica*. From Kellicott (Chordate Development). After Hall. A. Section through the eighth somite of an 8.5 mm. larva. B. Section through the mesonephric rudiment of a 25 mm. larva. C. Section through the pronephric chamber and the common nephrostome of the pronephros of a 25 mm. larva.

a. Dorsal aorta. *c.* Cœlom. *cn.* Common nephrostome. *g.* Germ cell. *i.* Inner tubule. *m.* Mesonephric rudiment. *my.* Myotome. *o.* Outer tubule. *p.* Remains of pronephros. *pc.* Posterior cardinal vein. *s.* Shelf cutting off the pronephric chamber from the remainder of the cœlom. *so.* Somatic mesoderm. *sp.* Splanchnic mesoderm. *W.* Wolffian duct. *I.* Primary mesonephric unit. *II.* Secondary mesonephric unit.

[4] Some authorities assert that the outer tubule probably never actually opens into the cavity of the primary unit from which it arises (Marshall, Hall).

ciliated as a typical *nephrostome,* this curious connection between body cavity and blood vessel persisting throughout life (Fig. 115, *F, G*).

The growth of these tubules has meanwhile been accompanied by a loss of the round or vesicular character of the region of the original primary unit. Thus between the point of origin of the secondary
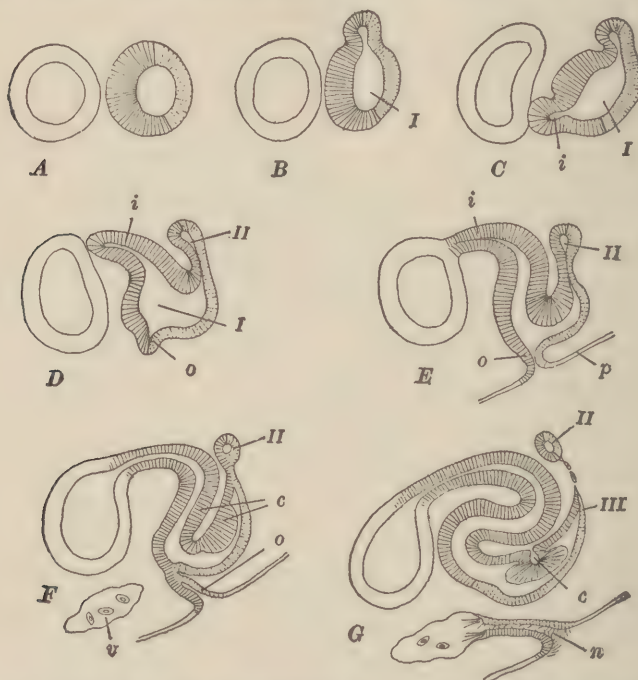
Fig. 115. — Series of diagrams illustrating the development of the primary meso-nephric tubules in *R. sylvatica.* From Kellicott (Chordate Development). After Hall. The Wolffian duct is drawn in outline simply. The mesonephric vesicles are shaded; the somatic part of the tubule is shaded by continuous lines, the splanchnic part by dotted lines. A. Wolffian duct and simple mesonephric vesicle. B. Mesonephric vesicle dividing into the large primary mesonephric unit and the small dorsal chamber. The latter elongates antero-posteriorly and represents the rudiment of the secondary and later mesonephric units. C. Formation of the rudiment of the inner tubule. D. Inner tubule extending upward and toward the mesonephric duct; formation of rudiment of outer tubule. E. Outer tubule fused with peritoneum and rudiment of nephrostome thus established. Bowman's capsule forming. Commencement of differentiation of secondary mesonephric unit. F. Separation of nephrostomal rudi-ment from remainder of tubule. G. Connection of nephrostome with branch of pos-terior cardinal vein. Separation of secondary unit, and beginning of tertiary unit indicated.

c. Bowman's capsule. *i.* Inner tubule. *n.* Nephrostome. *o.* Outer tubule. *p.* Peri-toneum. *v.* Branch of posterior cardinal vein. *I.* Primary mesonephric unit. *II.* Sec-ondary mesonephric unit. *III.* Tertiary mesonephric unit.

unit and that of the inner tubule, this region has become stretched out, and at the same time invaginated in a ventro-medial direction (Fig. 115, *E, F, G*). In this manner a cavity is produced which is later filled by a mass of capillaries connected with the dorsal aorta. This mass is essentially similar in character to the glomus of the pronephros, but in this case is termed the *glomerulus*, and of course has no connection with the cœlom. The surrounding walls of the invaginated unit in which the glomerulus thus lies embedded then constitute *Bowman's capsule*, the capsule and capillaries together being termed a *renal corpuscle* or *Malpighian body*.

The occurrence of similar processes in the other units finally results in a mass of tubules, glomeruli, and nephrostomes, which constitute the adult *mesonephric organ* or *kidney*. This organ is virtually complete by the time metamorphosis is ended.

THE ADRENALS

Though in no sense a part of the excretory system, these organs always occur in such close connection with the kidneys that it seems best to describe them at this point. Indeed, in the mature Frog the relationship of the adrenals and kidneys is more intimate than in the higher Vertebrates, so much so that it is difficult to separate them. Thus in this animal, the former organs appear merely as an area of
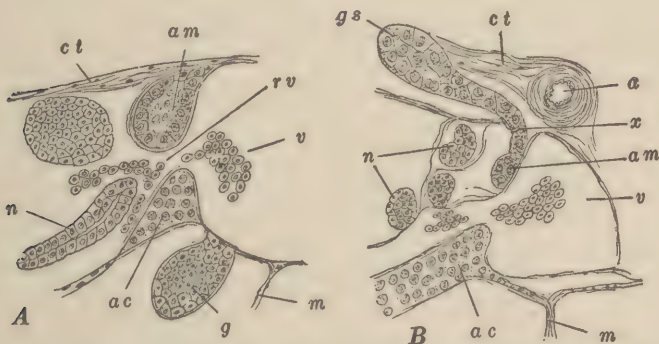
Fig. 116. — Parts of sections through young *R. temporaria,* showing the origin of the adrenal bodies. From Kellicott (Chordate Development). After Srdinko. A. Through 30 mm. tadpole. B. Through 11 mm. Frog after metamorphosis.

a. Dorsal aorta. *ac*. Corticle cells of adrenal body. *am*. Medullary cells of adrenal body. *ct*. Connective tissue. *g*. Gonad. *gs*. Sympathetic ganglion. *m*. Mesentery. *n*. Mesonephros. *rv*. Revehent renal vein. *v*. Vena cava. *x*. Point where ganglion cells enter mesonephros and adrenal body.

thin yellowish tissue attached to the ventral side of the mesonephros. They are composed, however, of two kinds of cells, the so-called *medullary substance*, and the *cortical substance*, which originate as follows:

The cortical substance is so named from the fact that in higher forms it occurs on the outside of the organ, though this is not true of the Frog. Here it consists of anastamosing cells apparently derived (at about 12 mm.) from the peritoneum in the neighborhood of the cardinal veins. These cells form a meshwork into which branches from the veins soon penetrate. The medullary substance consists of pigmented cells which appear later. They are derived originally from the sympathetic ganglia of the mesonephric region, and become scattered throughout the cortical tissue (Fig. 116).

INTERNAL DEVELOPMENT: THE GENITAL SYSTEM

The Gonoducts

In the Male. — The *vas deferens* of the Frog is simply the mesonephric or Wolffian duct, which serves as both ureter and sperm duct. Posteriorly, in the region of the cloaca, each duct develops a glandular *seminal vesicle*. Anteriorly, some of the tubules of the kidney give rise, in the regions of their Malpighian bodies, to strands of cells (the *rete cords*) which grow into the testes. These strands then become tubular and thus act as *vasa efferentia*.

At about 20 mm., there appears on each side of the cœlomic wall just beneath the pronephric region, a longitudinal thickening of the peritoneum. Along the dorsal border of this thickening there is then proliferated a ridge of cells, whose edge grows downward and presently fuses with the ventral border of the thickening. In this manner a tube is formed, which, when completed, is held close to the body wall by a thin covering of the general peritoneum (Fig. 117). The above process then continues anteriorly to a point opposite the base of the lungs and posteriorly to the cloaca, which it reaches subsequent to metamorphosis. In the male this tube develops no further, and is very inconspicuous and without function. It is the rudiment, however, of a *Müllerian duct* (see below).

In the Female. — The mesonephric duct is of course present in the female, but in this case acts only as a ureter. It possesses, never-

theless, extremely slight enlargements, representing rudimentary seminal vesicles. Vestigial vasa efferentia likewise occur in the female, forming a structure known as *Bidder's organ.*

Each Müllerian duct or *oviduct,* on the other hand, develops as described in the male, but does not stop at the point there indicated. Instead, the rudimentary duct moves away from the body wall somewhat, though it still remains attached to that wall by its peritoneal covering. Between the duct and the wall the two layers of the covering then fuse to form the mesentery-like sheet supporting the

Fig. 117. — Sections through the developing Müllerian duct of a **34 mm.** tadpole of *R. sylvatica.* From Kellicott (Chordate Development). After Hall. A. Section passing through the beginning of the Müllerian evagination. B. Section posterior to A. Duct established but still connected with peritoneum. C. Section still farther posterior, showing the separation of the duct from the peritoneum with which, however, it is covered.

M. Müllerian duct. *p.* Peritoneum. *t.* Third pronephric tubule.

oviduct. Anteriorly the duct turns down slightly, and its end becomes dilated as the *infundibulum,* while posteriorly it acquires an opening into the cloaca; between these points it gradually becomes greatly convoluted and thickened.

THE GONADS

The Indifferent Period. — As the early stages of these organs are identical in the male and female, a single account will suffice for both.

At about the time of hatching, a slight median dorsal ridge appears on the outside of the enteron (Fig. 118, *A*). It is composed of primordial germ cells, which, as in other cases, have apparently arisen from among the cells of the gut. Indeed, at this time it is difficult to dis-

tinguish the cells of the ridge from those which surround them. Presently, as noted above, the lateral plates of mesoderm press in toward each other in this region, and as they meet, they separate the ridge of cells (*sex-cell ridge*) from the enteron, so that the former lies just dorsal to the newly formed mesentery (Fig. 118, *B*). This ridge, now the *sex-cell cord* (not to be confused with the sexual cords), soon
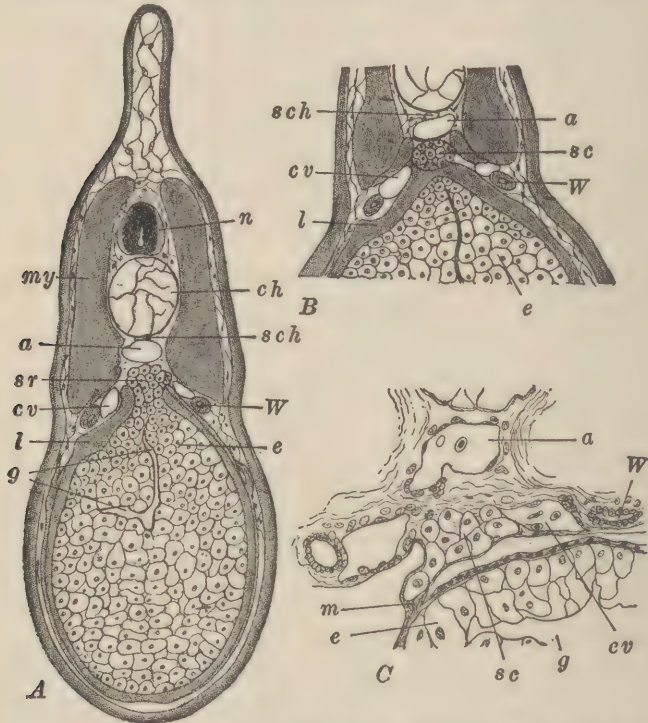
Fig. 118. — Sections showing the origin of the sex-cells (germ cells) in *R. sylvatica*. From Kellicott (Chordate Development). After Allen. A, B. Sections of a 7.5 mm. larva showing (A) sex-cell ridge of endoderm and (B) its separation as the sex-cell cord. C. Part of a section of an 8.3 mm. larva showing the beginning of the migration of the sex-cells, resulting shortly in the division of the sex-cell cord into two parts.

a. Dorsal aorta. *ch.* Notochord. *cv.* Posterior cardinal vein. *e.* Endoderm cells. *g.* Gut cavity. *l.* Lateral plate of mesoderm. *m.* Mesentery. *my.* Myotome. *n.* Nerve cord. *sc.* Sex-cell cord (not to be confused with sexual cords). *sch.* Subchordal rod (hypochorda). *sr.* Sex-cell ridge. *W.* Wolffian duct.

divides in two longitudinally and each part moves a short distance ventro-laterally, taking up its position just beneath one of the cardinal veins. The two parts covered by cœlomic epithelium (peritoneum)

project slightly into the cœlom in these regions and are known as the *genital ridges*.

The original primordial germ cells now begin to multiply while their number is apparently further augmented by the addition of cells from the epithelium which covers the ridges. In the meantime mesenchymal cells wander in among the germ cells forming the *stroma* or supporting tissue of the organ. Each ovary or testis is thus caused to project into the body cavity in which it is suspended by the peritoneal epithe-

lium which covers it. This epithelium gradually presses in above the organ, and thus forms a double sheet of tissue similar to that which supports the oviduct. As noted in the description of the adult organ, this sheet in the case of the ovary is termed the *mesovarium* and in the case of the testis the *mesorchium*.

The Period of Sexual Differentiation. — Meanwhile by the time the tadpole has reached a length of about 30 mm. (Bouin), the sexes begin to become differentiated (Fig. 119). In both the male and female the germ cells are arranged in nests, but in the ovary, the oögonia, as they may now be called, are not all alike. In each nest one of them presently increases in size at the expense of the other cells of the nest, and as is usual in such cases continues its development to form a mature ovum; the cells which surround it on the other hand, flatten themselves against it in a characteristic fashion and constitute its *follicle*. During each sea-

Fig. 119. — Section through the gonad of a 35 mm. tadpole of *R. temporaria*. From Kellicott (Chordate Development). After Bouin.

f. Follicle cells. *g.* Primitive germ cell. *m.* Mesentery. *n.* " Nests " formed by multiplication of the primitive germ cells. *s.* Genital strand (sexual cord) perhaps really rete cord.

son throughout the sexual life of the Frog, new oögonia and follicle cells arise from the epithelium in a similar manner.

In the testis there is also at the start a mass of similar cells in each nest, but in this case all are destined to mature as germ cells; i.e., spermatozoa. During their growth period the young sperm are arranged in bundles, and their heads are imbedded in the nurse cells lining the walls of the tubules which make up the testis. These tubules

have developed from the sexual cords, which in turn are probably
derived from the germinal epithelium. The sperm in some cases are
produced almost continuously; in others, they, like the ova, develop
only during the sexual seasons. In either case they also rise from the
indifferent epithelial cells of the organ.

In both sexes the anterior third or half
of each genital ridge fails to develop, as in-
dicated above. Instead, some time previous
to metamorphosis this portion of the organs
starts to become converted into the *fat bodies*.
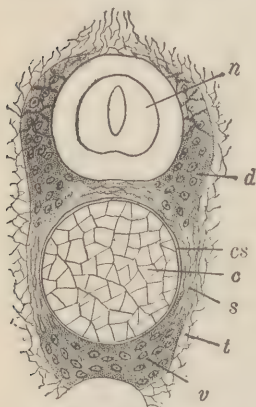
Fig. 120. — Transverse
section through the verte-
bral column in the body
region of a larva of *Xeno-
pus capensis*. From Kelli-
cott (Chordate Develop-
ment). After Schauinsland.
c. Notochord. *d.* Dorsal
vertebral cartilaginous arch.
s. Sclerotomal (skeletogen-
ous) sheath. *n.* Nerve cord.
cs. Chorda Sheath (primary
and secondary). *t.* Peri-
chondrial connective tissue.
v. Ventral (hypochordal)
vertebral cartilage. The
dorsal and ventral carti-
laginous elements have not
yet come to surround the
notochord.

INTERNAL DEVELOPMENT:
THE SKELETON

Only the briefest outline of this system
will be given, the reader being referred for
details to the more extensive works cited in
the bibliography.

THE VERTEBRAL COLUMN

At or a little before the time of hatching,
the skeletogenous sheath has already come to
surround the notochord and nerve cord, as
indicated above. Some time after hatching
(about 15 mm.), cartilage develops within
this sheath and presently becomes divided
into sections corresponding in position and
number to the future vertebræ. Within each
such section, moreover, the cartilage about
the chorda soon forms a ring which com-
pletely surrounds it (Fig. 120). Within
these cartilaginous rings, ossification now
starts and gradually spreads inward until the notochord at the core
of every ring is entirely obliterated. Thus is formed the *centrum*
of each vertebra. Meanwhile between these vertebral centra the noto-
chord is also obliterated by the ingrowth of cartilage. Each inter-
vertebral disc thus developed, later splits into an anterior and a
posterior part. Finally, during metamorphosis each of these parts
becomes ossified and fused with the end of the contiguous centrum.

In a similar manner the *neural arches* develop by ossification of the cartilage which extends dorsally from the centra around the nerve cord. Likewise the *transverse processes* arise as bits of cartilage which project laterally from each centrum, and later become converted into bone. Eventually minute cartilaginous ribs also develop at the ends of these processes, but are soon fused with the latter so as to be indistinguishable.

As already noted, the Frog possesses only nine real vertebræ, and the above description applies only to them. The skeletogenous elements
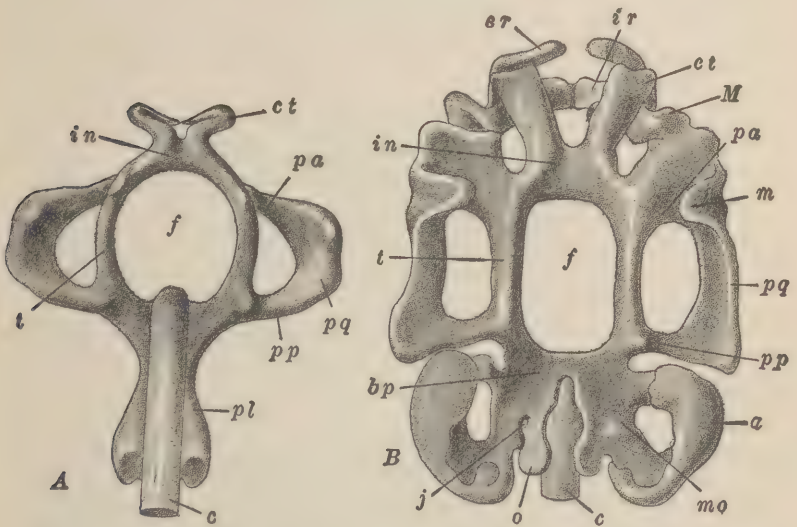
Fig. 121. — Dorsal views of the chondrocranium of the Frog larva. A. Chondrocranium of a 7.5 mm. larva of *R. temporaria*. From Kellicott (Chordate Development). After Gaupp, from Stöhr–Ziegler model. B. Chondrocranium of a 14 mm. larva of *R. fusca*. After Gaupp, from Ziegler model.

a. Auditory capsule. *bp*. Basal plate. *c*. Notochord. *ct*. Trabecular cornu. *f*. Basicranial fontanelle. *in*. Internasal plate. *ir*. Infrarostral cartilage. *j*. Jugular foramen (for IX and X cranial nerves). *m*. Muscular process. *M*. Meckel's cartilage. *mo*. Mesotic cartilage. *o*. Occipital process. *pa*. Anterior ascending process of palatoquadrate cartilage. *pl*. Parachordal plate. *pp*. Posterior ascending process of palatoquadrate cartilage. *pq*. Palato-quadrate cartilage. *sr*. Suprarostral cartilage. *t*. Trabecular cartilage.

of the last two somites, however, form a single tubular piece of cartilage which surrounds the end of the notochord. Later it also becomes mostly ossified, and is known as the *urostyle*.
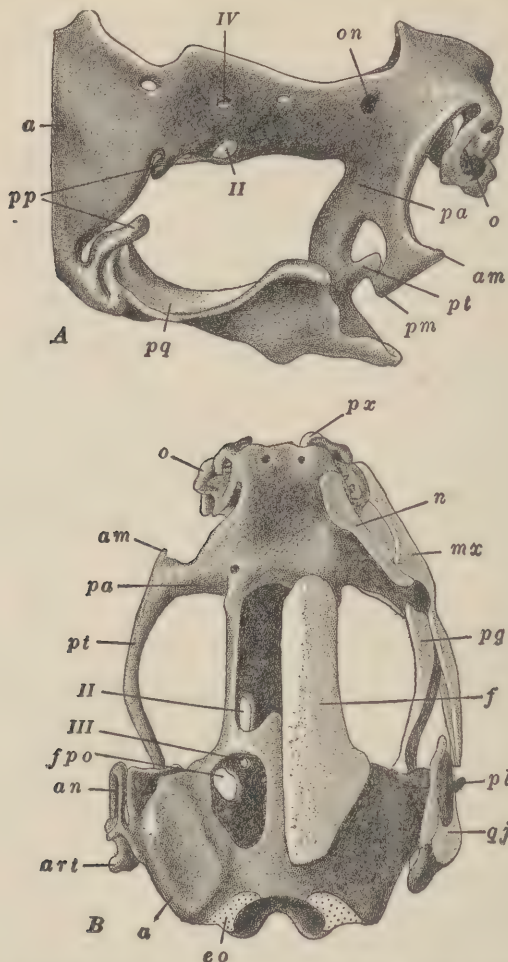
Fig. 122. — A. Anterior portion of chondrocranium of *R. fusca* during metamorphosis. Lateral view. From Kellicott (Chordate Development). After Gaupp, from Ziegler. B. Skull of a 2 cm. *R. fusca*, after metamorphosis. Dorsal view. Membrane bones removed from left side. After Gaupp, from Ziegler.

a. Auditory capsule. *am.* Anterior maxillary process. *an.* Annulus tympanicus. *art.* Articular process of palato-quadrate cartilage. *eo.* Exoccipital bone. *f.* Fronto-parietal bone. *fpo.* Proötic foramen. *mx.* Maxillary bone. *n.* Nasal bone. *o.* Olfactory cartilages. *on.* Orbito-nasal foramen. *pa.* Anterior ascending process of palato-quadrate. *pg.* Pterygoid bone. *pl.* Plectrum. *pm.* Posterior maxillary process. *pp.* Posterior ascending process of palato-quadrate. *pq.* Palato-quadrate cartilage. *pt.* Pterygoid process of palato-quadrate. *px.* Premaxillary bone. *qj.* Quadrato-jugal bone. *II.* Foramen for optic nerve. *III.* Foramen for III cranial nerve. *IV.* Foramen for IV cranial nerve.

THE SKULL

The Floor. — The posterior portion of the skull floor; i.e., that part which lies beneath the hind brain, is formed medially by the notochord. On each side of the notochord a cartilaginous rod develops which fuses with the chorda or rather with the cartilage which soon takes its place, thus completing the floor in this region. These rods are called the *parachordals*, and the fused mass is the *parachordal plate* (Fig. 121, *A*).

In front of each parachordal is another rod. These rods are curved somewhat, with their concave sides facing each other, and their pos-
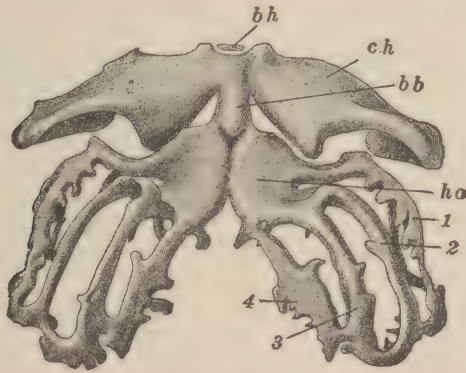
Fig. 123. — Hyoid and branchial arches of a 29 mm. larva of *R. fusca.* Ventral view. From Kellicott (Chordate Development). After Gaupp, from Ziegler.
bb. Basibranchial (first), or copula. *bh.* Basihyal. *ch.* Ceratohyal. *ho.* Hypobranchial plate. 1–4. First to fourth ceratobranchials.

terior ends fused with the anterior ends of the parachordals. Their own anterior ends grow toward each other and fuse between the olfactory organs; these rods are the *trabeculæ.* The space between them in the anterior floor of the skull is the *basicranial fontanelle,* which temporarily lodges the infundibulum. Later as the trabeculæ grow together this opening is closed.

The Sides, End, and Roof. — The floor has reached the stage indicated only a short time after hatching. The other cartilaginous parts of the skull then develop as follows:

In the posterior region the auditory capsules appear at the sides of the head (Fig. 121, *B*). Ventrally they are presently united with the

skull floor by the *mesotic* and *occipital* cartilages. The capsules thus
form the sides of the posterior part of the skull, while the occipital
cartilages grow up to form the posterior walls and the roof of this
region. Between the occipitals is a posterior opening, the *foramen
magnum*, through which the spinal cord passes in to the brain.

Anteriorly the trabeculæ grow up to form the sides of the skull
in the neighborhood of the orbits. Their more anterior portions then
grow together dorsally forming the anterior roof. Between this an-
terior roof and the posterior one formed by the occipitals is the *supra-*
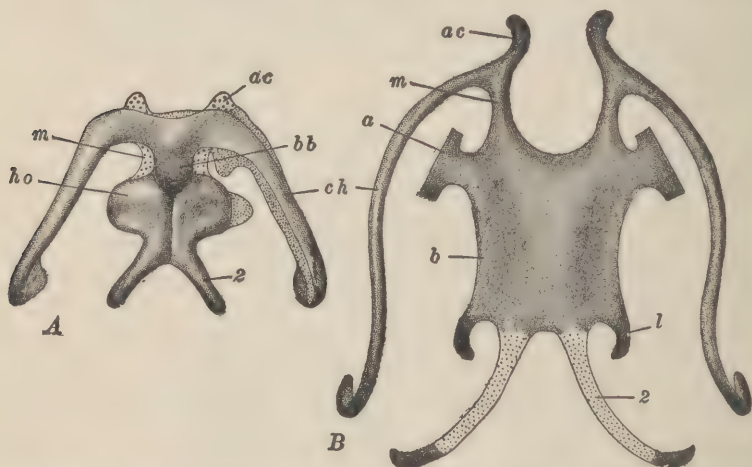
Fig. 124. — A. Hyobranchial apparatus of *R. fusca*, toward the end of metamor-
phosis. The left side is shown in a more advanced stage than the right, in that less
cartilage is present. The original cartilage is indicated by fine stipples. The coarse
stipples indicate the cartilage added during the early part of metamorphosis. From
Kellicott (Chordate Development). After Gaupp, from Ziegler. B. Hyobranchial
apparatus of a 2 cm. *R. fusca*, after metamorphosis. After Gaupp, from Ziegler.
a. Alar process. *ac.* Anterior process of hyoid cornu. *b.* Body of hyobranchial
cartilage. *bb.* Basibranchial (first), or copula. *ch.* Ceratohyal (hyoid cornu in B).
ho. Hypobranchial plate. *l.* Postero-lateral process of hypobranchial cartilage. *m.*
Manubrium. *2.* Remains of second ceratobranchial (postero-medial process of
hyobranchial cartilage).

cranial fontanelle. The extreme anterior ends of the trabeculæ go to
form the *olfactory capsules*, which are partly separated from the
brain cavity by a septum. All of these changes, both anterior and
posterior, are virtually completed in larvæ of 3 cms.

Dermal Elements in the Skull. — The cartilaginous skull thus
formed later becomes ossified. Before this occurs, however (about
20 mm.), many of the parts begin to be covered by bony plates origi-

nating in the dermis and called *dermal bones* (Fig. 122). Some of these plates, such as the *fronto-parietals*, serve to cover open spaces left in the cartilage; e.g., the supra-cranial fontanelle. Most of the dermal bones as well as those formed in the cartilage have appeared before metamorphosis is complete.

The Visceral Arches. — These arches at first consist merely of concentrations of mesoderm, as indicated above. Shortly after the mouth opens, however, all have developed skeletal elements of cartilage. The cartilage of the mandibular arch early becomes divided into a dorsal portion, the *palato-quadrate,* and a ventral portion, *Meckel's cartilage*. The former becomes fused anteriorly and posteriorly with the trabeculæ and at metamorphosis is considerably modified to form a part of the upper jaw. As noted above, furthermore, a small outgrowth becomes separated from the posterior or quadrate portion of this cartilage and gives rise to the annulus tympanicus of the middle ear. Meckel's cartilage remains small throughout larval life, but constitutes the core of the lower jaw in the adult.

Part of the first and second branchials, together with certain median elements, form the *hypobranchial apparatus* of the adult. A portion of the hyoid arch (*ceratohyal*), moreover, persists in connection with this apparatus as the *hyoid cornu.* All of the other arches disappear entirely at metamorphosis (Figs. 123, 124).

The Appendicular Skeleton

The *pectoral girdle,* with the exception of the clavicle, is formed from ossified cartilages, the clavicle being dermal in origin. The *pelvic girdle* and the bones of the limbs all arise from ossification in cartilage.

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PART III

THE TELEOSTS AND GYMNOPHIONA

THE TELEOSTS AND GYMNOPHIONA: THEIR SEGMENTATION AND GASTRULATION

Before beginning the study of the Chick, it is desirable to give a very brief account of the processes of segmentation and gastrulation in the Teleosts (Bony Fishes) and the Gymnophiona. It is of advantage to understand these processes in the forms mentioned because embryologically they are intermediate between those found in the Frog and those in the Reptile or Bird; i.e., the Sauropsids. This of course is not meant to imply that *modern* Fishes, Amphibians, and Sauropsids form a direct phylogenetic series. It is merely suggestive in a general way of the manner in which the embryological conditions in the lower forms have apparently been modified in the process of evolution.

THE TELEOSTS

SEGMENTATION

In the Frog the yolk is more or less concentrated in the vegetal half of the egg, but is not sufficiently dense to prevent the whole egg from segmenting. In the Teleosts, on the contrary, the concentration of yolk is very marked, so that the protoplasm exists only as a thin plate upon the animal pole. As noted in Chapter II, this plate is called the *blastodisc,* and from it the entire embryo arises, the remainder of the egg being purely nutritive. In these eggs, therefore, when segmentation begins, the process is confined to this disc, and is said to be *meroblastic* or *discoidal,* as opposed to the holoblastic or total cleavage of Amphioxus and the Frog (Fig. 124A).

The first two planes of division pass entirely through the disc and at right angles to one another. The segmentation then becomes more or less irregular, the disc soon being entirely cut up into cells, whereupon it is called the *blastoderm.* Between the latter and the yolk, a

space has meanwhile developed, which is termed the *segmentation cavity*, and which corresponds to the cavity of the same name in the Frog (Fig. 124B). Thus the egg has become a *blastula*.

In the yolk around the margin of the blastoderm are a number of nuclei (*yolk nuclei*) which are derived partly from the blastodermal edge, and partly perhaps from extra sperm (merocytes). These nuclei presently begin to divide amitotically, and become amœboid, in some
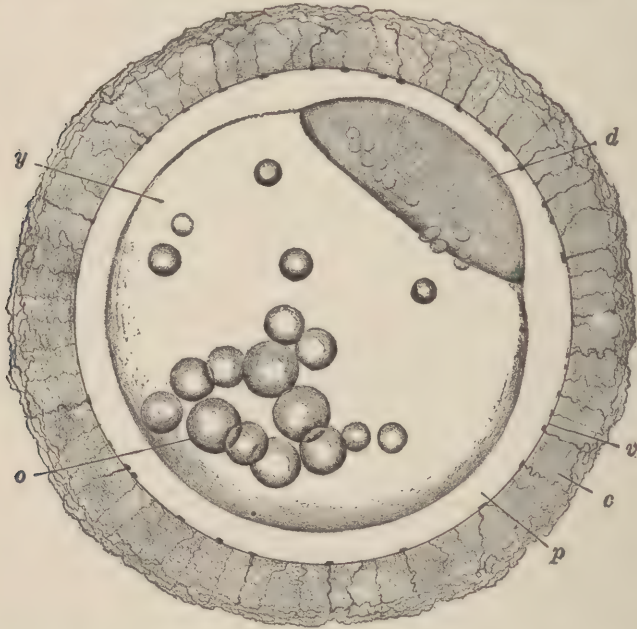
Fig. 124A. — Egg of the Teleost, *Fundulus heteroclitus*. From Kellicott (General Embryology). Total view, about an hour after fertilization.
c. Chorion. d. Protoplasmic germ disc or blastodisc. o. Oil vacuoles. p. Perivitelline space. v. Vitelline membrane. y. Yolk.

cases migrating centrally beneath the blastoderm. Here they occupy the thin layer of protoplasm which forms the floor of the segmentation cavity, giving the latter the character of a syncytium. This syncytium or *periblast*, as it is termed, presently spreads over the entire yolk, and is perhaps instrumental in making the latter assimilable by the cells of the blastoderm. At all events, it finally disappears without taking any part in the formation of the actual embryo; hence it need not be considered further.

GASTRULATION

Involution. — In that region of the blastoderm which is destined to form the posterior end of the animal, the blastodermal rim now begins to turn under; i.e., is involuted. Thus, in this region a lower layer of cells begins to be formed which spreads anteriorly into the segmentation cavity beneath the blastoderm. This second layer is the primordial *endoderm,* which is destined later to give to the de-
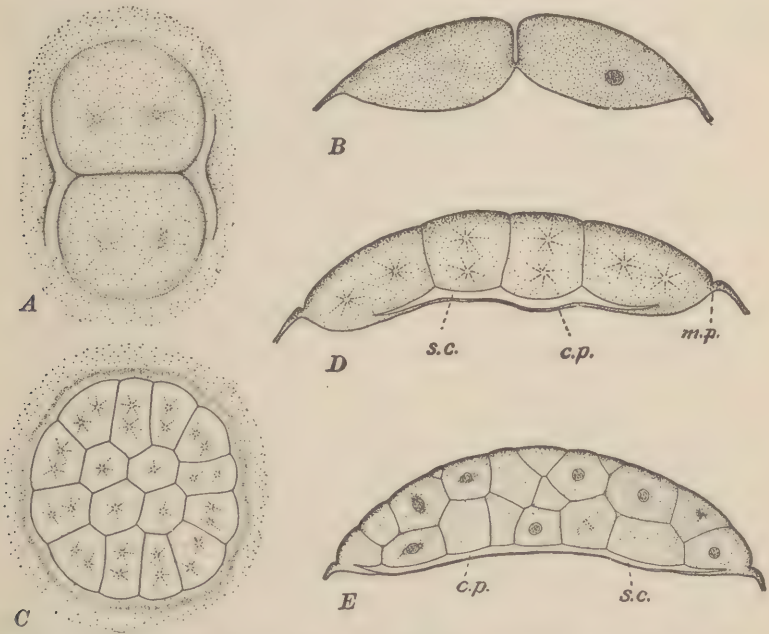
Fig. 124B. — Cleavage in the sea-bass, *Serranus atrarius.* From H. V. Wilson. A. Surface view of blastodisc in the two-cell stage. B. Vertical section through the four-cell stage. C. Surface view of blastodisc of sixteen cells. D. Vertical section through sixteen-cell stage. E. Vertical section through the late cleavage stage.
c.p. Central periblast. *m.p.* Marginal periblast. *s.c.* Segmentation cavity (blastocœl).

finitive or gut endoderm, the notochord, and the mesoderm. The upper layer, on the other hand, may now be called *ectoderm,* while the margin of the blastoderm where the involution is taking place constitutes the dorsal lip of the blastopore (Fig. 125). As the endoderm spreads out within the former segmentation cavity, the latter becomes thereby transformed into the *archenteron.* The new cavity, like its predecessor,

is obviously extremely shallow, and though roofed by the endoderm is open below to the surface of the yolk, or more strictly speaking to the thin syncytial layer of periblast. Lastly, it is to be noted that while the process of involution is most active at the posterior edge of the blastoderm, it is also occurring to a much lesser degree all around the margin.

Epiboly. — While involution is thus progressing chiefly at the posterior edge of the blastoderm, very active epiboly is taking place about the remainder of the blastodermal margin. The latter process is most rapid at the anterior edge and decreases gradually along either side,
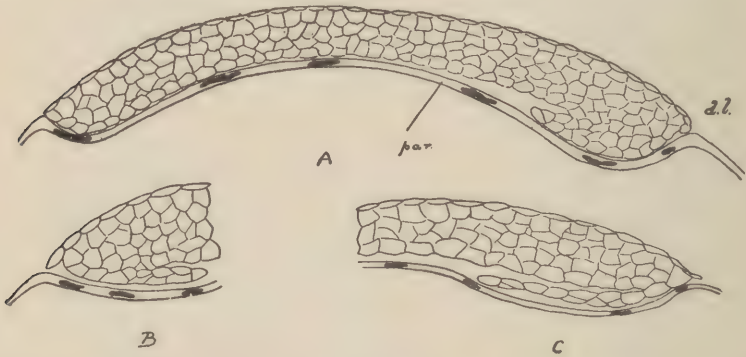
Fig. 125. — Sagittal sections through the blastoderm of *Serranus* during the formation of the germinal layers. From Jenkinson (Vertebrate Embryology). After H. V. Wilson. A. Beginning of involution and slight epiboly at dorsal lip (*d.l.*). B. Epiboly at anterior edge. C. Further progress of involution at dorsal lip.
  d.l. Dorsal lip. *par.* parablast (periblast).

until at the posterior edge it is in most cases comparatively slight. The result of these movements is then obviously to envelop the entire yolk with a covering of cells, the *yolk-sac.* Furthermore, as suggested in Chapter II, it is possible in eggs of this type to regard the entire rim of the blastoderm as really homologous with the *blastoporal lip.* Hence while the posterior edge is recognized as the dorsal lip, the lateral edges must be regarded as the lateral lips and the anterior edge as the ventral lip. This homology becomes more obvious when, in company with the above envelopment of the yolk, the anterior blastodermal edge passes around the vegetal pole and up on the posterior side, thus becoming a ventral lip in position as well as in name (Fig. 126). This means of course that the completion of the process

of epiboly involves likewise the closure of the blastopore. Moreover, because of the relatively great distance traversed by the ventral lip as compared with the dorsal, the point of closure is well up on the posterior side of the yolk. In fact in many instances the epiboly of the dorsal lip is so slight that the closure occurs fairly close to the region where the involution of this lip began.
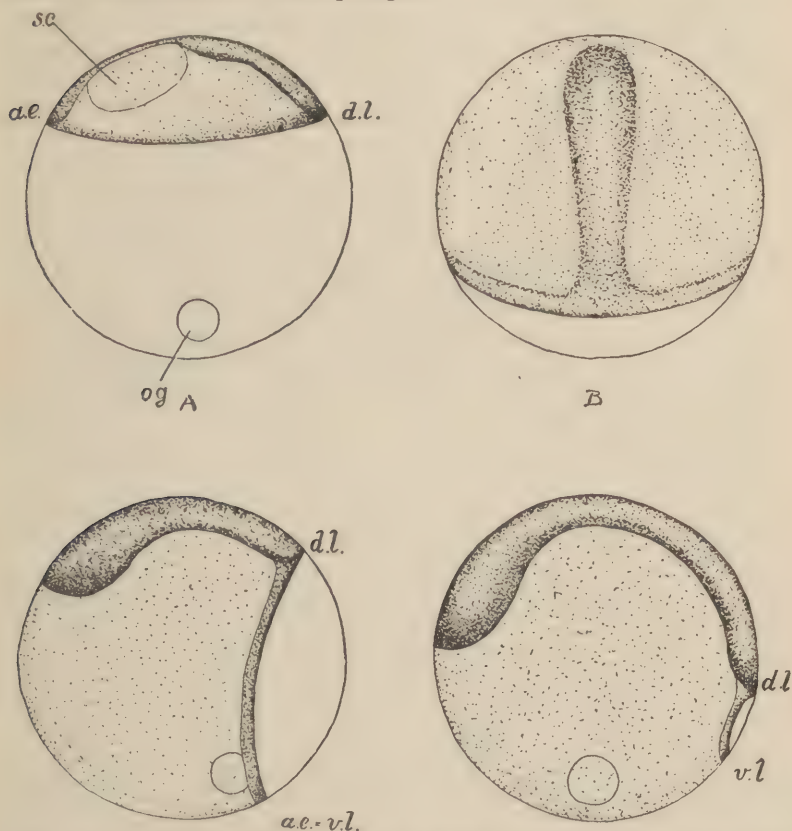
Fig. 126. — Growth of the blastoderm over the yolk (epiboly) after the formation of the material for the embryo in the Teleostan fish *Serranus*. From Jenkinson (Vertebrate Embryology). After H. V. Wilson.

d.l. Dorsal lip of the blastopore (posterior edge of the blastoderm). *a.e.* Anterior edge of the blastoderm or ventral lip (*v.l.*) of the blastopore. *s.c.* Segmentation cavity. *o.g.* Oil-globule.

Concrescence. — During the process of epiboly just described, there appears also to be a certain amount of concrescence, or flowing to-

gether of the lateral lips along the median line of the future embryo. The degree to which this occurs, however, apparently varies somewhat in different Fish, and is probably not so great in any of them as was once supposed. Thus in the Sea Bass (H. V. Wilson), it would seem that only a very small portion of the lateral lips fuse in the manner indicated, the main body of the embryo arising from the blastoderm anterior to the point where the fusion begins. Posterior to this point the confluence produces a thickened line of cells extending backward for a relatively short space. At approximately the anterior end of this line is *Kupffer's vesicle,* a cavity which is supposed to represent in part a rudiment of the neurenteric canal, the latter feature never
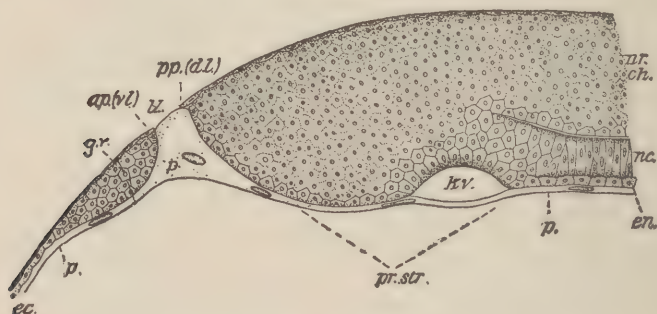
Fig. 127. — Sagittal section through the hinder end of a Fish embryo (*Serranus*), showing the undifferentiated primitive streak, anterior to which the structures of the embryo are being differentiated. From H. V. Wilson.

a.p. (*v.l.*). Anterior margin of the blastoderm or ventral lip of blastopore, after having grown entirely around the yolk mass. *bl.* Blastopore. *ec.* Ectoderm. *en.* Endoderm. *g.r.* Germ ring. *k.v.* Kupffer's vesicle. *nc.* Notochord. *nr. ch.* Nerve cord. *p.* Periblast. *pp.* (*d.l.*). Posterior margin of blastoderm (dorsal lip of blastopore). *pr. str.* Primitive streak.

actually developing as such in the Teleosts (Fig. 127). At the posterior extremity of the line of confluence, upon the other hand, is the spot where concrescence is brought to an end by the completion of the process of epiboly; i.e., the point where the *blastopore* closes. This point in the Fish is not marked by a proctodæal invagination, as in the case of the Frog, and hence it is impossible to say certainly whether the spot of closure exactly coincides with the place where the anus subsequently opens. Nevertheless, since this spot is later brought forward beneath the endoderm of the gut, it seems probable that the position of the anus and the place of blastoporal closure do approximately coincide. Upon this basis, however, it is evident that the thick-

ened line of cells indicated above occupies the same relative position as the primitive streak in Amphioxus and the Frog; i.e., between the neurenteric canal (or Kupffer's vesicle) and the anus. Likewise, as in the forms previously studied, it is formed by the fusion of a portion of the lateral lips of the blastopore. Hence in the Teleosts also this region may be properly termed the *primitive streak*. The mass of cells in and around the more posterior portion of it, however, because of their character and future history, are often designated as the *caudal knob* (Fig. 127). Thus is produced the Teleostean *gastrula*.

Comparison of the Blastoporal Lips in the Teleost with Those in Preceding Forms. — There now remain to be emphasized certain differences between the behavior of the blastoporal lips in such extremely telolecithal eggs as those of the Teleosts and in eggs with less yolk. Thus in Amphioxus and the Frog, the lateral lips of the blastopore for the most part suffer concrescence and so furnish material to the axial region of the embryo. It is only their remnants lying farthest from the original dorsal lip whose union at the very end of gastrulation closes the blastopore and at the same time forms the primitive streak. In eggs such as those of the Teleosts, on the other hand, concrescence, as suggested above, apparently occurs to only a limited extent. This may well be due to the fact that since so much of the material of the lips is used in the process of covering the relatively huge yolk mass, little is left to contribute to the embryo by means of concrescence. At all events, whatever the cause of the limited amount of this process, its slight occurrence is correlated with two other features as follows: First, relatively less of the blastoporal lips go into the embryo than in the case of eggs with less yolk, and more, possibly, into the formation of the primitive streak. Second, as a corollary of this, those parts of the lateral lips which form the streak in the Teleost are the parts of the original blastodermal margin lying relatively near to the most posterior point of that margin (dorsal lip) at the beginning of gastrulation. This obviously is just the opposite of the situation where there is a large amount of concrescence. Finally in this connection it may be noted that in the Teleosts, as in the preceding forms, the term *germ ring* is generally made synonymous with the lips of the blastopore; i.e., with the entire blastodermal rim. However, where, as in extremely telolecithal eggs, only a small portion of the blastodermal rim actually contributes material to the embryo, it is a question whether the phrase germ ring is really applicable to the entire rim. In such cases it would

perhaps be more accurate to confine the use of the term to only that part of the rim or blastoporal lip which is in fact involved in concrescence.[1]

THE DIFFERENTATION OF MESODERM, NOTOCHORD, AND DEFINITIVE ENDODERM

It has been stated that involution occurred chiefly at the dorsal lip of the blastopore. The result is that in the region anterior to this lip beneath the roof of the archenteron, there is an extensive double layer of cells produced by this process. From the dorsal side of the lower or involuted of these two layers (primordial endoderm), between
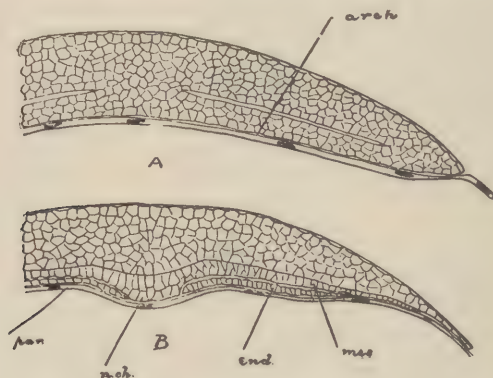
Fig. 128. — Transverse sections through the differentiating blastoderm of *Serranus* showing differentiation of the roof of the archenteron into notochord (*nch.*), mesoderm (*mes.*), and endoderm (*end.*). *par.* Parablast (periblast). From Jenkinson (Vertebrate Embryology). After H. V. Wilson.

it and the overlying ectoderm, the *mesoderm* is now delaminated in two sheets situated upon either side of the middle line (Fig. 128). Presently also the primordial endoderm along the middle line itself becomes separated from that upon either hand, and is aggregated into an axial rod, the *notochord*, with the sheets of mesoderm upon each side of it

[1] It should nevertheless be added with respect to this point that even in such eggs (e.g., those of certain Fish) it has been found possible by proper stimulation to cause the development of an embryo in relation to any point on the blastodermal margin (Stockard). Hence it would appear that in any case the entire margin must be regarded as at least *potentially* germ ring. Of course, in the event that it were decided to employ the term in the limited sense indicated above, it would be understood that where only a partial circumference is concerned the word " ring " could no longer be used literally.

(Figs. 128, 129). What remains of the original primordial endoderm may now be spoken of as actual or definitive endoderm, destined to form the lining of the gut. Since, however, the formation of the notochord consumed all of the primordial cells along its line of origin, the definitive endoderm consists for a short time of two separate lateral sheets. Shortly, these sheets unite with one another beneath the notochord, and the enteric roof is thus again complete.

It remains to be noted that the mesoderm thus set aside in the position indicated above, may be called axial or *gastral*. Posteriorly it passes without interruption into the undifferentiated line of tissue which is arising from the concrescence of the blastoporal lips to form the
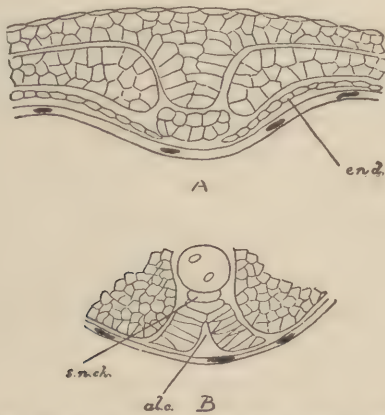
Fig. 129. — Formation of the gut (*al.c.*) in *Serranus* by the bending down of the sides of the roof of the archenteron. In A note also the nerve cord forming by a solid invagination of ectoderm (characteristic of many Teleosts) instead of by folds. From Jenkinson (Vertebrate Embryology). After H. V. Wilson.

s.n.ch. Sub-notochordal rod. *end.* Endoderm.

primitive streak, and possibly in certain cases some of the axial material immediately anterior to this. In so far, then, as the cells within the streak and the axial material just indicated later give rise to mesoderm, this mesoderm (or the cells from which it arose) while still within the blastoporal lips, may be said to have been *peristomial*. From this it is evident that the relationship of the two types of mesoderm; i.e., gastral and peristomial, is not different from what it was in Amphioxus and the Frog. The gastral mesoderm is distributed along the axis of the embryo, and as fast as the peristomial tissue comes into this position and is actually differentiated into mesoderm, it too be-

comes gastral. From these considerations, however, one thing is obvious; i.e., that the less the concrescence in any case, the less the amount of peristomial mesoderm which can be thus transformed.

EARLY FORMATION OF THE EMBRYO

As soon as the germ layers are formed in the embryonic region of the blastoderm, and while the remainder of the latter is still in the process of enclosing the yolk, the outlines of the embryo begin to become clearly evident. This is the result of a folding off process by which the embryo is gradually raised above the surface of the yolk. Since, moreover, the folding involves all three cell layers, it is evident that the innermost layer, or endoderm, will become bent downward and eventually inward along either side of the middle line so as to form the rudiment of a tube — the primitive gut (Fig. 129). By a continuation of this folding, and also by rapid growth in all directions, the embryo soon comes to extend outward above the yolk-sac which is attached like a bag to its ventral side. The tail, it may be noted, is largely formed by outgrowth from the caudal knob.

THE GYMNOPHIONA

SEGMENTATION

Segmentation in these somewhat aberrant Amphibians is again virtually meroblastic, and hence results in the formation of a *blastula* with a *blastoderm* and *segmentation cavity* very similar to that of the Teleost. In this case, it is true, there is a slight superficial cleavage in the yolk which forms the floor of the cavity, and this also extends out around the periphery of the blastoderm. The bulk of the yolk, nevertheless, remains undivided.

GASTRULATION

Involution and Epiboly. — The advent of gastrulation becomes evident by the occurrence of involution and epiboly at what proves to be the posterior edge of the blastoderm; i.e., the dorsal blastoporal lip. Both processes take place together in this region and thus there is produced an outer layer of *ectoderm,* and an inner layer of primor-

dial *endoderm* spreading out within the segmentation cavity above the partially segmented yolk. The method is made evident by reference to the median longitudinal sections of the blastoderm in figure 130, *A* and *B*. Up to this point, it will be noted, the movements observed are not essentially different from those which took place at a corre-
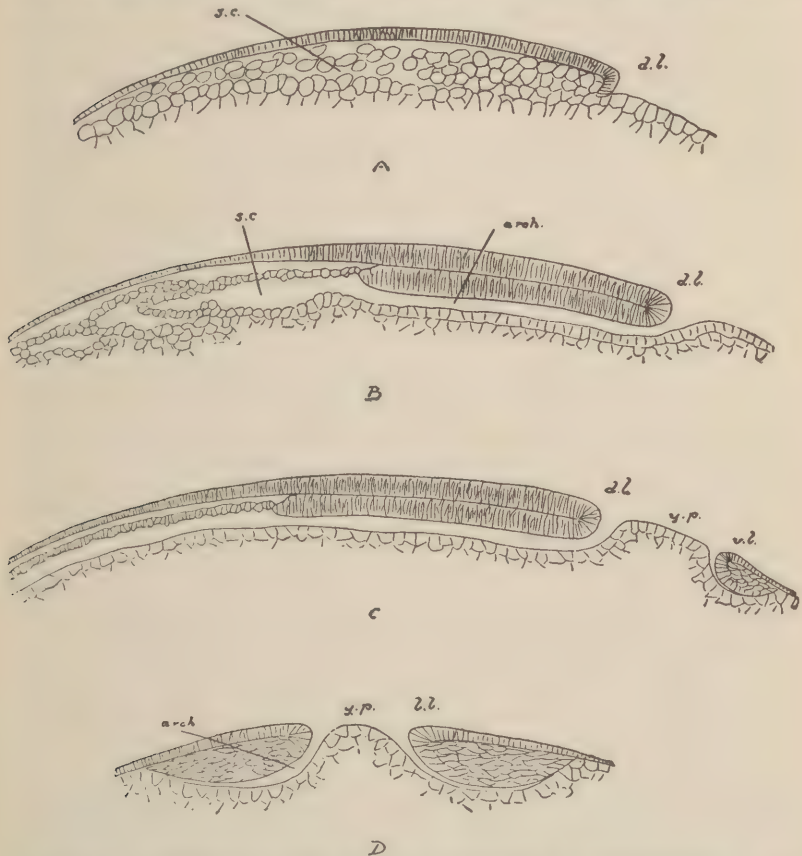
Fig. 130. — Formation of the germ layers in *Hypogeophis* (a Gymnophionian). From Jenkinson (Vertebrate Embryology). After Brauer. A–C. Sagittal sections of three successive stages. D. Transverse section through the blastopore and yolk plug (*y.p.*). *s.c.* Segmentation cavity into which in B and C the archenteron (*arch.*) opens. *d.l.* Dorsal lip. *l.l.* Lateral lip. *v.l.* Ventral lip.

sponding stage in the Fish. The points in which the gastrulation of the Gymnophiona digresses from that in the forms thus far studied and resembles that in the Birds, remain, therefore, to be noted.

The Gymnophionian Blastopore. — A surface view of the blasto-derm as gastrulation commences (Fig. 131), will reveal the fact that the posterior portion of the rim where involution is occurring has the shape of a wide crescent, whose ends or horns bend backward. As the process goes on, moreover, these horns continue to grow posteriorly, and presently turn toward one another until they meet (Fig. 131, B, C, D). It is furthermore to be noted that this movement has
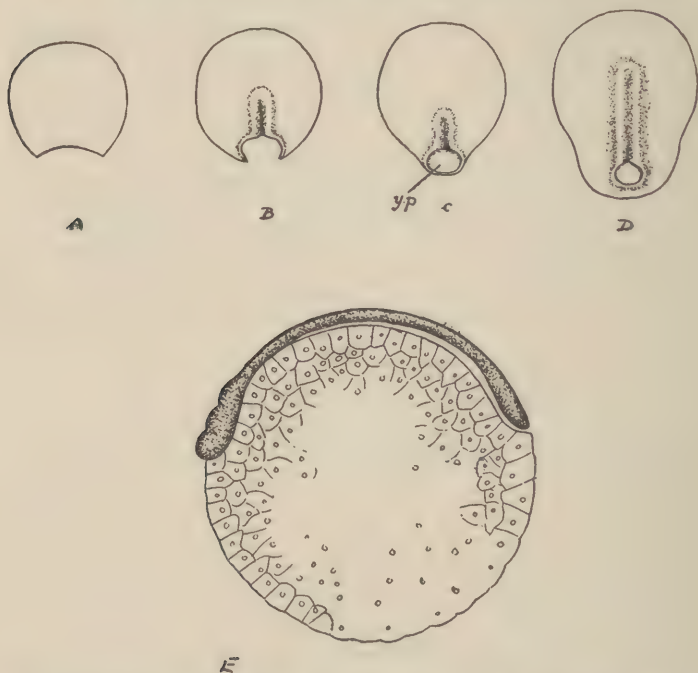
Fig. 131. — Formation and closure of the blastopore in the Gymnophiona. From Jenkinson (Vertebrate Embryology). A–D. Surface views of the blastoderm of *Hypogeophis*. After Brauer. The lateral lips are seen to meet behind and so form the ventral lip. *y.p.* Yolk plug. E. Embryo of *Ichthyophis* lying on the partially seg-mented yolk which is still uncovered by the blastoderm. After the brothers Sarasin.

occurred relatively rapidly, whereas the epiboly of the anterior side of the blastoderm, so rapid in the Fish, has scarcely started. The results of these processes compared with those in the Teleosts, as well as with those in forms with less yolk, may now be stated as follows:

If the entire blastodermal rim is still regarded as the *lip of the blastopore* (germ ring), it must be said that the movements just noted

have divided this lip into two portions. One of these is quite limited; i.e., it merely furnishes the boundary for the small area of yolk (*yolk plug*) enclosed by the fused horns of the crescent (Fig. 131, *C*). The second portion of the original lip, on the other hand, bounds the entire remaining expanse of uncovered yolk. Moreover, since epiboly has been slight, this expanse comprises almost as much yolk surface as existed prior to the beginning of gastrulation. Such is the situation thus far indicated. Upon the basis of subsequent development, however, it may be stated that the small area enclosed by the horns of the crescent is the only part which really corresponds to the blastopore in the forms previously studied. Hence, as would be expected, its ultimate closure gives rise to a line of tissue quite homologous with the typical *primitive streak,* the *neurenteric canal* arising at its anterior end and the *anus* at the other. From this it appears that in the Gymnophiona, the anterior and most of the lateral parts of the blastodermal rim take no part in forming the ventral and lateral lips of the region which must be homologized with a true blastopore; these lips are formed by the horns of the crescent. Instead, the outer (anterior and most of the lateral) portions of the rim are occupied merely with the gradual covering of the main body of the yolk, long after the true blastopore has been definitely delimited. Under such circumstances it is evident that any concrescence from the sides of the blastodermal rim must occur before the fusion of the horns of the crescent has taken place. Since this fusion is effected quite early, however, it seems unlikely that the above process is of much significance in the embryology of this group.

It may now be emphasized again that the results just described are brought about by the relative slowness of epiboly on the part of the anterior and lateral portions of the blastoderm. The parts immediately about the posterior edge are, on the other hand, more active, and so bring about the formation of a separate blastopore and its relatively early closure. These features especially should be kept clearly in mind as being extremely similar in their fundamental aspects to those which will be encountered in the Bird. With them as a basis it should then be possible readily to relate the peculiar characteristics of avian gastrulation to those already noted in the Fish, the Frog, and Amphioxus.

The Differentiation of Mesoderm, Notochord, and Definitive Endoderm

By means of the above processes of epiboly and involution, there is presently developed a telolecithal *gastrula*, whose lower or endodermal layer forms a roof for the former segmentation cavity (now the *archenteron*) in much the same way as in the Teleosts. In the present case, also, this layer soon gives rise to the *mesoderm* and *notochord*. The latter originates quite as in the Fish, but the formation of the mesoderm differs in one respect. Thus in the Teleost it will be recalled that, though the development of the notochord involved all of the
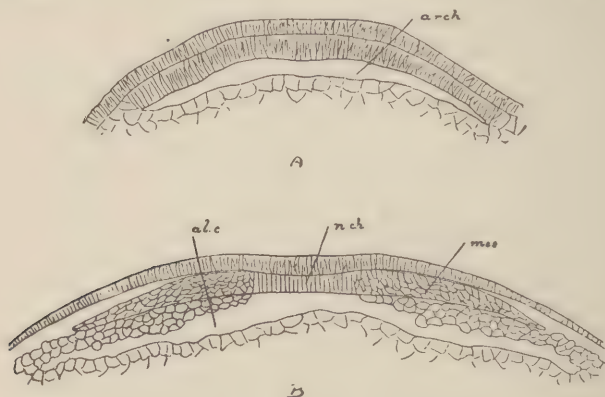
Fig. 132. — Transverse sections of *Hypogeophis* showing the differentiation of the roof of the archenteron into notochord (*n.ch.*) and mesoderm and the formation of the gut (*al.c.*) by upgrowth of yolk-cells from the sides. From Jenkinson (Vertebrate Embryology). After Brauer.

primordial endoderm in the median line of the embryo, the mesoderm on either side was merely split off, leaving a layer of definitive endoderm beneath it. In the Gymnophionian, on the other hand, the entire central portion of the archenteric roof which did not go to form the notochord becomes mesoderm (Fig. 132). There is no delamination, and the result is that within the central area of the blastoderm, the enteric cavity for the time being is roofed only by mesoderm and notochord. In other words, in this case the central portion of the mesoderm, as well as the notochord, consumes in its formation all of the primordial endoderm beneath it. Presently, however, the defini-

tive endoderm in this central region is supplied by the ingrowth of lower layer cells from about the margin (Fig. 132). As in the forms previously studied all three layers are continuous with one another about the lips of the blastopore.

As will presently appear, the methods of mesoderm and notochord formation, as just described in the Teleosts and Gymnophiona, are not particularly significant as regards an understanding of these processes in the Bird. Nevertheless, because of the fact that, as usual, their occurrence somewhat overlaps the process of gastrulation as strictly defined, an account of their character in the present forms has been included for the sake of completeness.

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CHAPTER VII

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PART IV

THE DEVELOPMENT OF THE CHICK

THE CHICK: THE ADULT REPRODUCTIVE ORGANS, AND THE DEVELOPMENT OF THE EGG PREVIOUS TO INCUBATION

The Chick has long been an object of embryological interest, the study of its development being connected with such classical names as Malpighi (1672), Wolff (1759), and Von Baer (1828). In the more modern era of science, moreover, workers in this field have continued to study it, until at the present time probably more details regarding its development are known than in the case of any other animal. As will appear, however, certain points concerning the very early stages are even yet in doubt, and are still under investigation.

Some of the reasons for the importance of this form and the study which has been given it may be briefly indicated. In the first place the material is usually easy to obtain and observe throughout most of the developmental stages. Furthermore, unlike the Frog or Fish, the Chick embryo, in common with those of other Birds as well as with those of Reptiles, possesses certain very significant extra-embryonic membranes and appendages. The significance of these structures, moreover, lies not only in their character and functions in the groups just cited, but also in the fact that the same appendages and membranes occur also in the Mammals, though in a somewhat modified condition. Lastly, aside from the features already indicated, the general development of the Chick is more nearly mammalian than that of any of the forms previously considered.

In the following account we shall begin with a brief description of the reproductive organs of the adult Bird.

REPRODUCTIVE ORGANS OF THE ADULT, OÖGENESIS AND OVULATION

THE MALE

The male Bird, or Cock, possesses a pair of testes, each of which is an ellipsoidal body about two inches long and one inch in diameter.

It is made up of the *seminiferous tubules* and supporting tissue, and, as in the case of the Frog, is rather closely attached to the dorsal wall of the cœlom by a fold of cœlomic epithelium, the *mesorchium*. By way of the *vasa efferentia,* each testis discharges its products into its respective *vas deferens*. The latter duct then leads to the cloaca, where its entrance is marked by a papilla.

THE FEMALE

The Ovary. — In the embryo Chick two ovaries are present, but only the left develops. In the adult Fowl this is suspended from the body wall by the *mesovarium* in about the same position as the left testis in the male. It consists of the usual vascular connective tissue

Fig. 133. — Section of an ovarian ovum of the Pigeon, drawn from a preparation of Mr. J. T. Patterson. From Lillie (Development of the Chick). The actual dimensions of the ovum are 1.44 x 1.25 mm.

f.s. Stalk of follicle. *G.V.* Germinal vesicle. *Gr.* Granulosa. *L.* Latebra. *p.P.* Peripheral protoplasm. *pr.f.* Primordial follicles. *Th.ex.* Theca externa. *Th.int.* Theca interna. *Y.Y.* Yellow yolk. *Z.r.* Zona radiata.
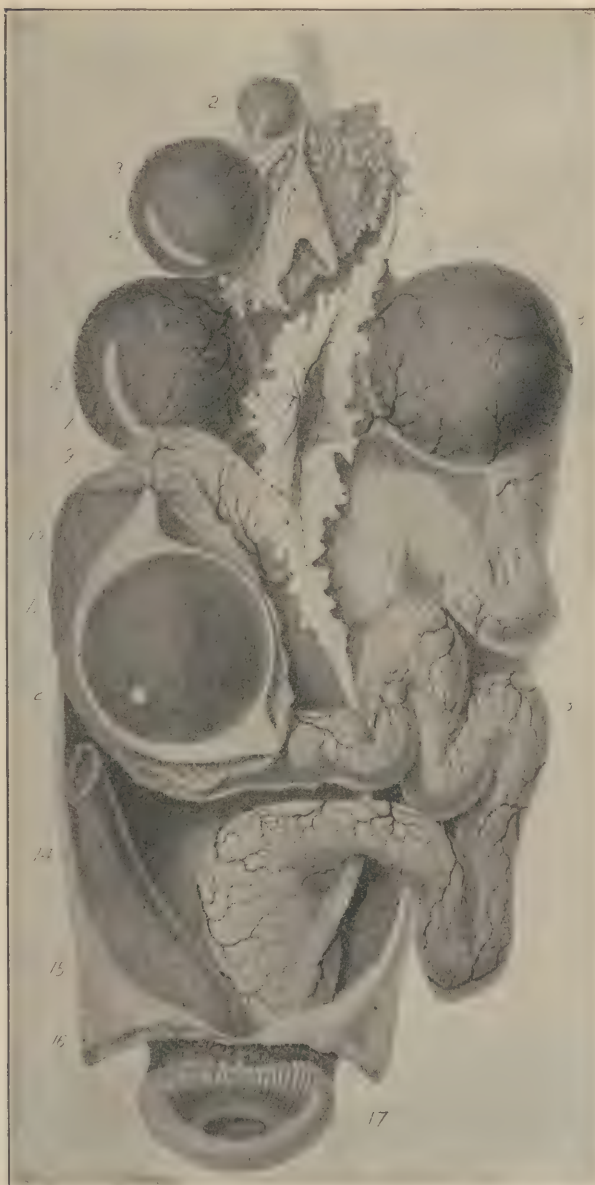
Fig. 134. — Reproductive organs of the Hen. (After Duval, based on a figure by Coste). From Lillie (Development of the Chick). The figure is diagrammatic in one respect, namely, that two ova are shown in the oviduct at different levels; normally but one ovum is found in the oviduct at a time.

1. Ovary; region of young follicles. 2 and 3. Successively larger follicles. 4. Stigmata (cicatrices), or non-vascular areas, along which the rupture of the follicles takes place. 5. Empty follicle. 6. Cephalic lip of ostium. 7. Funnel of oviduct (ostium tubae abdominale). 8. Ovum in the upper part of the oviduct. 9. Region of the oviduct in which the albumen is secreted. 10. Albumen surrounding an ovum. 11. Ovum in portion of duct laid open to show it. 12. Germinal disc. 13. Region of the oviduct in which the superficial layers of albumen and the shell-membrane are formed. 14. Lower part of the oviduct ("uterus," shell gland). 15. Rectum. 16. Reflected wall of the abdomen. 17. Anus, or external opening of cloaca.

elements, or *stroma*, within which are imbedded ova in various stages of growth. Each ovum is surrounded by a layer of *follicle* or *granulosa* cells, and these in turn are encased in a sheath of the stroma called the *theca*. It is sometimes customary to refer to such eggs together with their coverings as simply *follicles* (Fig. 133). Normally only one ovum matures at a time, though there may be several not many hours apart.

The Genital Tract. — As in the case of the ovary, only the left genital tract develops. It is a much convoluted tube which opens anteriorly in the neighborhood of the ovary and posteriorly into the cloaca just dorsal to the anus. It is suspended from the dorsal body wall by a special mesentery-like fold of peritoneum, and may be divided into three main parts, as follows:

I. The Oviduct Proper. This is the anterior part and is itself divisible into three sections:

(a) *The Infundibulum or Ostium.* This is a thin-walled muscular funnel, the inner surface of which is lined by ciliated epithelium. It is in the immediate neighborhood of the ovary, but does not directly connect with it.

(b) *The Glandular Portion.* This is a long much convoluted tube following immediately after the ostium. It leads into:

(c) *The Isthmus.* This is a shorter tube also glandular whose posterior end marks the termination of the oviduct proper.

II. The Uterus. This is a relatively short, dilated portion whose walls are also glandular. It immediately follows the isthmus and leads into the third and last main division:

III. The Vagina. This region is likewise short, but thin-walled, and opens into the cloaca (Fig. 134).

OÖGENESIS

The Oögonia. — The origin of the primordial germ cells and their multiplication as oögonia occur during the embryonic life of the Chick. This early history will therefore be dealt with later in connection with the development of the gonads. At the time of hatching, however, the oögonia have ceased to divide, and each is becoming surrounded by follicle cells preparatory to growth (Fig. 135). They may now, therefore, be called oöcytes, or young ova, whose history from this point onward will be taken up in more detail.

The Growth Period.

The Vitelline Membrane or Zona Radiata. — Each ovum or oöcyte now has secreted about it a membrane which is called the *vitelline membrane*. Whether it is a true vitelline membrane arising entirely from the surface of the egg itself, or whether it is secreted by the
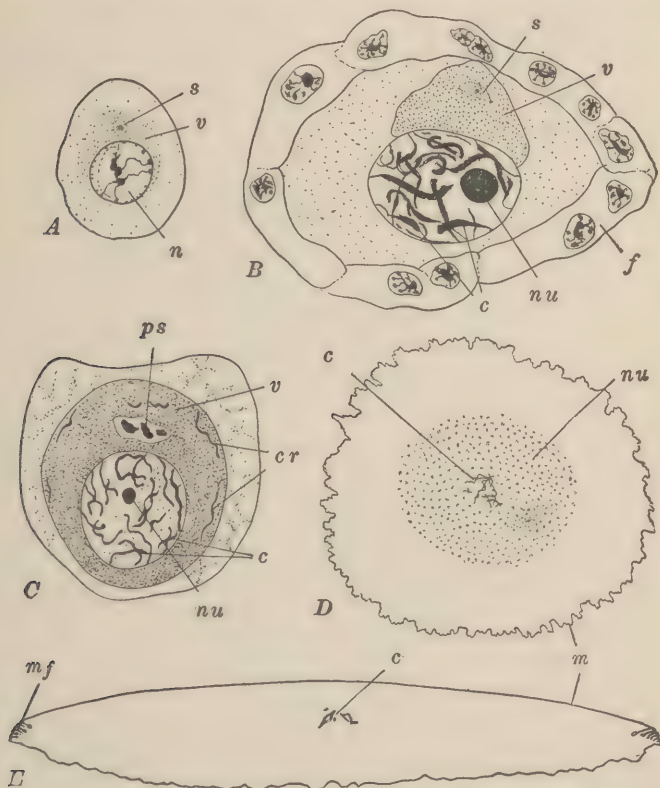
Fig. 135. — Growth stages in the oögenesis of the Hen's egg. From Kellicott (Chordate Development). After Sonnenbrodt. A. Oöcyte measuring 0.012 x 0.016 mm., the nucleus of which is 0.006 mm. in diameter. B. Oöcyte measuring 0.018 x 0.028 mm., the nucleus of which is 0.0105 x 0.014 mm. Enclosed in follicle. C. Oöcyte measuring 0.040 x 0.045 mm., the nucleus of which is 0.020 x 0.022 mm. D. The nucleus only, of an oöcyte measuring 5.84 x 6.16 mm., the nucleus itself measuring 0.214 x 0.238 mm. Total view showing the small chromosomes in the midst of a collection of chromatin nucleoli. E. Vertical section of the nucleus only, of an oöcyte, the follicle of which measured 37 mm. in diameter. The nucleus itself is 0.455 mm. in diameter and 0.072 mm. in greatest thickness.

c. Chromosomes. *cr.* Extra nuclear chromosome-like bodies. *f.* Follicle. *m.* Nuclear membrane. *mf.* Folds in nuclear membrane. *n.* Nucleus. *nu.* Chromatin nucleolus. *ps.* Pseudochromosomes. *s.* Centrosphere. *v.* Yolk nucleus or vitellogenous body.

follicle cells and is therefore chorionic in character, is somewhat uncertain. As this membrane thickens slightly, it becomes pierced by minute canals; for this reason it is also referred to sometimes as the *zona radiata*. Through these canals by way of the follicle cells the egg receives nourishment from the surrounding theca.

The Germinal Disc. — At first the nucleus occupies the center of the oöcyte, and the yolk granules are deposited in the cytoplasm around it.
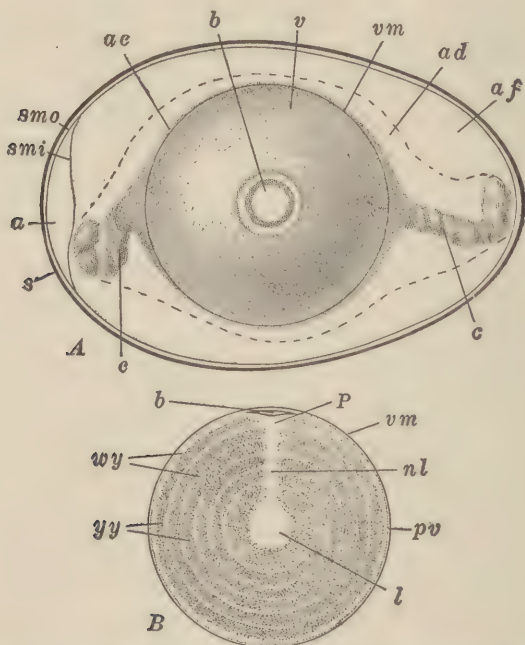
Fig. 136. — Semidiagrammatic illustration of the Hen's egg at the time of laying. From Kellicott (Chordate Development). A. Entire " egg." Modified from Marshall. B. Vertical section through the vitellus or ovum proper, showing the concentric layers of white and yellow yolk.

a. Air chamber. *ac.* Chalaziferous layer of albumen. *ad.* Dense layer of albumen. *af.* Fluid layer of albumen. *b.* Blastoderm. *c.* Chalaza. *l.* Latebra. *nl.* Neck of Latebra. *P.* Nucleus of Pander. *pv.* Perivitelline space. *s.* Shell. *smi.* Inner layer of shell membrane. *smo.* Outer layer of shell membrane. *v.* Vitellus or " yolk." *vm.* Vitelline membrane. *wy.* Layers of white yolk. *yy.* Layers of yellow yolk.

This presently results in the existence of yolk-free cytoplasm only around the periphery of the egg. This cytoplasm, however, is thicker upon the side where the theca of the ovum is attached to the ovary;

this thickening is called the *germinal disc (blastodisc)*. Meanwhile the ovum has been growing, and by the time it has become .6 mm. in diameter, the nucleus has migrated into this disc (Fig. 133).

The Deposition of Yolk. — The growth of the ovum is largely due to the deposits of yolk, which it appears occur in the following manner: The nucleus, as noted, occupies at first a central position around which the yolk begins to be formed. This yolk is of a lightish color termed *white yolk,* and the central mass of it which is thus deposited is known as the *latebra.* Following this the peripheral layer of the protoplasm starts to deposit around the latebra a darker colored substance, the *yellow yolk.* This deposit is not constant, but periodic, and for this reason the yellow yolk occurs in concentric layers with very thin sheets of a substance which appears to be white yolk between them. Furthermore, as the egg is thus enlarged, the nucleus, as indicated, leaves its central location and takes a peripheral position, which it maintains during subsequent growth. The result is that the concentric yellow layers are everywhere interrupted along the path which the nucleus has taken. Along this path there is thus left a continuous deposit of white yolk extending from the latebra almost to the surface. It is known as the *neck* of the latebra, and just beneath the blastodisc it spreads slightly to form a plate, the *nucleus of Pander.* At the completion of the growth process a final layer of white yolk is deposited over the entire surface of the egg save in the region of the blastodisc. It seems to arise from the original peripheral layer of protoplasm which has now disappeared (Fig. 136, *B*).

OVULATION

During these processes the nucleus has greatly enlarged and as usual in its enlarged form it is known as the *germinal vesicle*. The nuclear wall then disappears, and the spindle for the first maturation division forms. At about the time the latter event takes place, the fully grown ovum is grasped by the funnel-shaped infundibulum. The theca and follicle then rupture along a non-vascular line, known as the *cicatrix,* and the egg is received into the oviduct. Normally during each seasonal laying period of the hen, one ovum a day thus matures and is released from the ovary. Occasionally, however, two may be released together; in this case they are enclosed in a single shell and form a " double yolk egg." It seems likely also that the infundibulum

occasionally picks up an ovum which has fallen into the body cavity, the extra egg being then enclosed with the one received in the normal manner.

THE HISTORY OF THE OVUM FROM FERTILIZATION THROUGH GASTRULATION

The stages now to be described have not all been completely worked out for the Chick. It is presumed, however, that they are somewhat similar to the corresponding stages in the Pigeon which have been fully described by Patterson and Blount. Data concerning doubtful stages in the Hen's egg have therefore been partially supplied from the facts regarding the Pigeon. The points where this has been done will be noted in passing.

FERTILIZATION AND MATURATION

When the egg is taken into the ostium, it is at once surrounded by sperm which have been received from the male at a period from 24 hours to two weeks previous to the ovulation of the ovum in question. Several sperm enter the egg presumably, as in the Pigeon, in the neighborhood of the blastodisc, and previous to the first maturation division. A pause follows while both divisions are completed, and then a single sperm nucleus fuses with that of the egg. Many of the remaining sperm nuclei then degenerate, while others (*supernumerary nuclei* or *merocytes*) persist for a time and produce certain phenomena to be described later in connection with segmentation.

THE APPLICATION OF ALBUMEN, SHELL MEMBRANES, AND SHELL

Strictly speaking, the formation of the ovum proper is completed at the time of ovulation, and it thus appears that what is ordinarily spoken of as the " yolk " of the Hen's egg is really the entire egg. Nevertheless, in the case of the Bird, it is common usage to include under the term egg not only the ovum proper (i.e., the " yolk ") but also all its tertiary membranes, and this usage will be adhered to in the following account:

As the yolk passes down the oviduct it takes a position such that a line passing through the blastodisc and the center of the vegetal pole

is at right angles to the longitudinal axis of the duct at any particular point. It then revolves slowly about the latter axis, and while so doing receives its respective coverings from certain portions of the duct.

The Glandular Portion.

The Dense Albumen and Chalazæ. — The passage of the yolk through the glandular part of the oviduct requires about three hours, during which there is secreted about it a thick layer of *dense albumen*. Moreover, owing to the revolving movement, indicated above, this albumen is laid upon the egg in spiral sheets. It must be noted also that the innermost layer of this substance is especially dense. This layer thus forms a sort of thick membrane over the yolk, and extends from each side of it in short twisted cords, a little distance up and down the oviduct (Fig. 134). The membrane is the *chalaziferous membrane,* and the cords are the *chalazæ.* The direction of their application is such that in the completed egg one cord will extend toward the small end of the shell and the other toward the large end.

The Isthmus.

The Shell Membranes and Fluid Albumen. — The ovum goes through the isthmus of the duct in about an hour, and receives upon the dense albumen two thin *shell membranes.* In the completed egg these membranes lie immediately beneath the shell and are closely adherent to each other at every point except at the large end of the egg. Here they are slightly separated to form the *air space.* Outside of these membranes a *fluid albumen* is next secreted, which presently passes through them and thus comes to lie next to the dense albumen (Fig. 136, *A*).

The Uterus.

The Calcareous Shell, and Additional Fluid Albumen. — The egg takes from twelve to sixteen hours to pass through the uterus, and while doing so receives the *calcareous shell* formed of carbonates, and phosphates of calcium and magnesium. It also receives more fluid albumen which passes through the partly formed shell and shell membranes to unite with the fluid albumen already present.

The Vagina.

The egg is now completed and receives no addition while within this part of the duct. If it has reached this stage before 4 P. M., it may be

laid at once; in case its arrival occurs after 4 p. m., it will be retained until the following day. This fact accounts for the different stages of development of freshly laid eggs.

SEGMENTATION

While the egg has been passing down the oviduct and receiving its outer coverings, segmentation has been practically completed. As in the Teleost and Gymnophiona eggs, this process involves only the germinal disc (blastodisc), which at the time of the first cleavage is about 3 mm. in diameter and 0.5 mm. thick. It takes place in the following manner and in the parts of the duct indicated:

The First Cleavage. — The first cleavage furrow forms in about the middle of the blastodisc, and extends only part way across it and part way through it. It is completed during the passage of the glandular portion of the duct (Fig. 137, *A*).

The Second and Third Cleavages and the Accessory Cleavage. — As the egg enters the isthmus the second cleavage furrow begins to form in the two existing cells; it is approximately perpendicular to the middle of the first furrow, and is of about the same depth. There thus arise four cells, in each of which the furrow of the third cleavage soon appears. These third cleavage furrows may be parallel with the first, but their direction is quite frequently irregular. In this manner eight cells are formed, none of which are at first separated from the deeper protoplasm of the disc or from that at the margin.

Before continuing the account of the regular cleavages it is now necessary to pause a moment to note certain so-called *accessory cleavages*. These cleavages, which are extremely light and transitory in the Hen's egg, seem to result from a few divisions of some of the supernumerary sperm nuclei indicated above. They appear at about the four cell stage as faint radial furrows around the edge of the blastodisc, but by the time ten cells have formed they have completely vanished. Scattered and degenerating sperm nuclei are sometimes observable as late as the thirty-two cell stage; these also, however, are presently lost sight of, and apparently exercise no influence upon the ovum (Fig. 137).

The Central and Marginal Cells. — Subsequent to the eight cell condition, following the third cleavage, further furrows soon appear, which result in the production of approximately sixteen cells. Some

of these furrows, moreover, are such as definitely to bound the outer edges of those cells, whose protoplasm has heretofore been continuous with that which lay further out. Hence, there is thus created a central segmented area completely delineated from the unsegmented protoplasm about it; the cells of this area are termed the *central cells.*
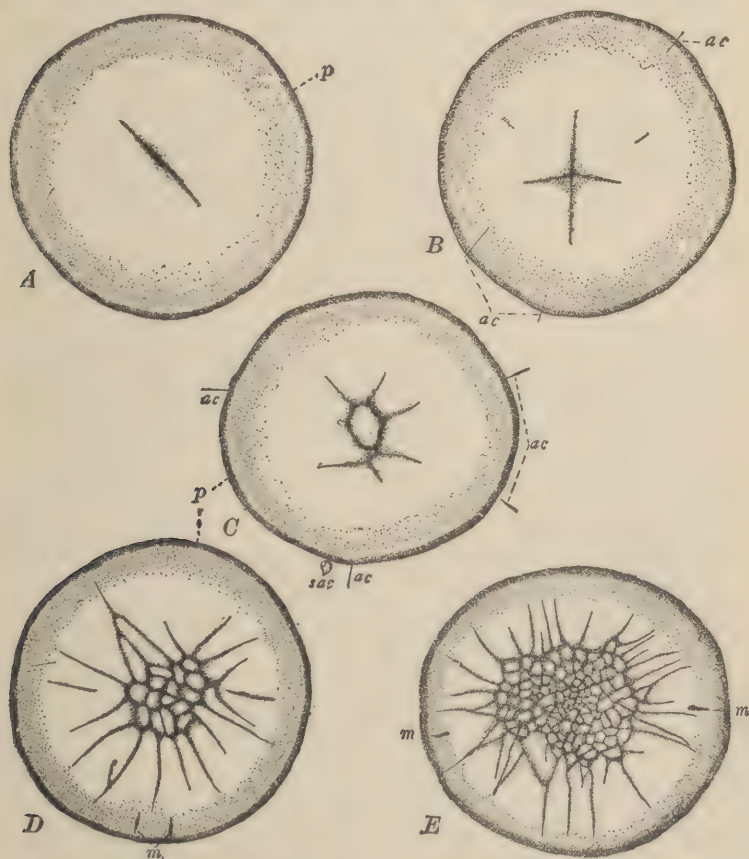
Fig. 137. — Cleavage in the Hen's egg. Surface views of the blastoderm and the inner part of the marginal periblast only. From Patterson. The anterior margin of the blastodisc is toward the top of the page. A. Two cell stage about three hours after fertilization. B. Four cells, about three and one-fourth hours after fertilization. C. Eight cells, about four hours after fertilization. D. Thirty-four cells, about four and three-fourths hours after fertilization. E. One hundred and fifty-four cells upon the surface; the blastoderm averages about three cells in thickness at this stage (about seven hours after fertilization).

ac. Accessory cleavage furrows. *m.* Radial furrows. *p.* Inner part of marginal periblast. *sac.* Small cell formed by the accessory cleavage furrows.

Cleavage then continues about the rim of this central area, and thus new cells appear here, which for a time are unseparated from the protoplasm lying still further toward the periphery. These new cells, because of their position, are called *marginal cells*. As the process of cleavage goes on they are constantly being cut off and added to the central cells; meanwhile beyond them more marginal cells arise. In this manner the central segmented area is continually increasing in diameter (Figs. 138, *A,* and 139).
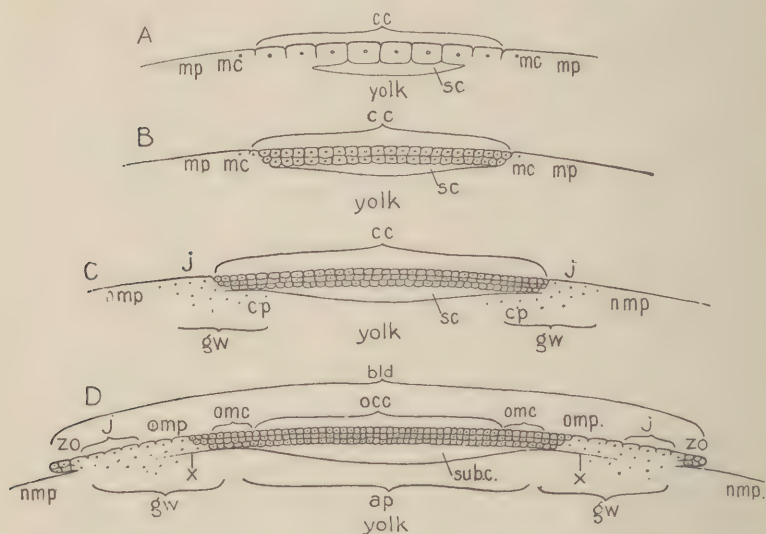
Fig. 138. — Diagrams of verticle sections through the blastoderm of a Hen's egg during cleavage stages. A. A section through an approximate 32 cell stage. B. A section through a slightly later stage where marginal cells are being added to the original central cells. C. A section through a still later stage in which the marginal cells have all been used up, and the extra (periblast) nuclei from some of them are invading the periblast to form the germ wall. D. A stage just as gastrulation is about to begin. The zones of junction and overgrowth are well marked, and the germ wall is beginning to add cells to the original marginal cells.

ap. Approximate extent of the area pellucida, not yet marked, however, by the thinning of the blastodermal roof. *bld*. Blastoderm. *cc*. Central cells. *cp*. Central periblast. *gw*. Germ wall. *j*. Zone of junction. *nmp*. New marginal periblast. *mc*. Marginal cells. *mp*. Marginal periblast. *occ*. Original central cell region. *omc*. Original marginal cell region. *omp*. Original marginal periblast region. *sc*. Segmentation cavity. *sub. c*. Subgerminal cavity. *x*. Line of separation between the inner portion of the germ wall and the underlying yolk. *zo*. Zone of overgrowth.

The Segmentation Cavity. — Furthermore, at the same time that the central cells are being defined as such by the furrows at their

margins, horizontal cleavages are also taking place. These cleavages intersect the furrows which are visible from the surface, and thus cut off a single superficial layer of the central cells from the proto-plasm beneath it. Fluid then begins to collect between this layer of cells and the protoplasm, thus establishing a shallow space, the rudi-ment of the *segmentation cavity.*
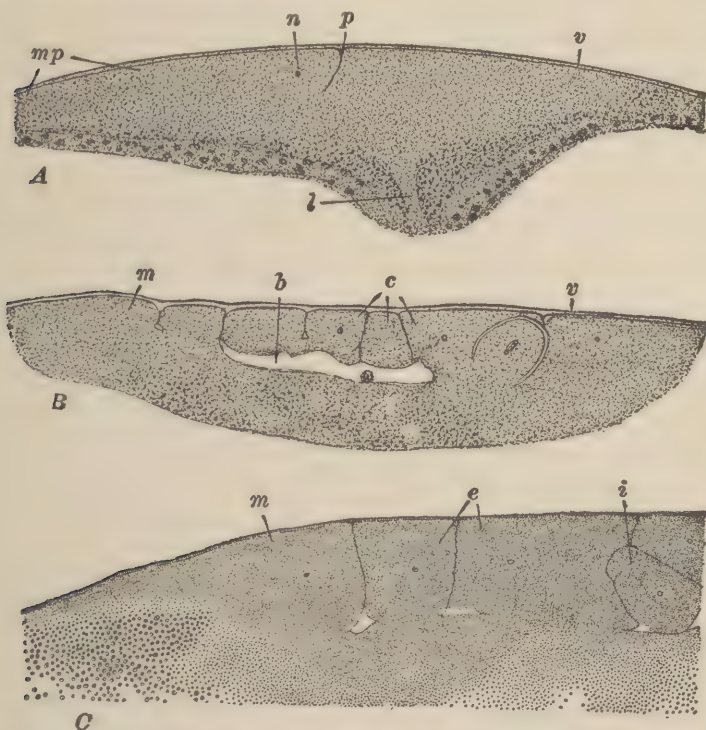
Fig. 139. — Verticle sections through the Chick blastoderm during the process of cleavage. From Kellicott (Chordate Development). After Patterson. A. Section through the two cell stage. B. Median section through the thirty-two cell stage. C. Part of a longitudinal section through the sixty-four cell stage.

b. Blastocœl or segmentation cavity. *c.* Central cells. *i.* Inner cell cut off by hori-zontal cleavage plane. *l.* Neck of latebra. *m.* Marginal cells. *mp.* Marginal periblast. *n.* Nucleus. *p.* First cleavage. *v.* Vitelline membrane.

As the egg leaves the isthmus, there have been formed in this manner approximately thirty-two cells [1]; it next enters the uterus, in which cleavage is completed and gastrulation begun.

[1] There are, according to Kölliker, about forty-four cells in the blastoderm of the Chick at this stage.

The Periblast and Its Segmentation. — Further division both horizontal and otherwise now takes place, so that the layer of central cells, at first only one cell thick, soon acquires a thickness of several cells; the area covered by the central and marginal cells has likewise been increased. All the cleavage thus far indicated, however, has taken place within the central region of the blastodisc (Fig. 138, *B*). About the margin of this area, there remains a ring of the disc slightly darker in color than the central portion, and about .5 mm. wide. It is still entirely unsegmented and is known as the *periblast*.

The Germ Wall and Subgerminal Cavity.[2] — Presently the formation of marginal cells about the edge of the central region reaches to the inner margin of the ring, defined as periblast. At this point, although the nuclei of the marginal cells continue to divide, the cytoplasmic cleavages do not keep pace with them. The extra nuclei (*periblast nuclei*) thus produced then wander out into the region of the periblast and convert it into a syncytium. Some of these nuclei even move centrally for a short distance into the unsegmented protoplasm beneath the rim of the segmentation cavity. The latter region of protoplasm thus occupied by the extra nuclei is usually known as the *central* or *subgerminal* periblast (see below), to distinguish it from the strictly marginal periblast, the two regions, however, being perfectly continuous. Following the above-mentioned penetration by the periblast nuclei, the two types of periblast together are henceforth known as the *germ wall* (Fig. 138, *C*). Meanwhile, the last of the original marginal cells have been cut off from the outlying periblast (now germ wall), and have become continuous with, and similar in character to, the cells originally defined as central. Within the syncytial germ wall, cytoplasmic cleavage next begins to take place, and the cells which are thus produced are added to the former marginal cells. Thus, partly by the multiplication of the cells already in existence, and partly by the peripheral addition of new cells arising within the wall, the central area of completely defined cells spreads outward over the surface of the yolk. Upon this basis it might be imagined that the germ wall would soon be used up, and as regards the portion of it defined as central periblast this appears to be true. That part of the wall designated as marginal periblast, however, is

[2] The ensuing description of the organization of the periblast and other later phases of segmentation are from the accounts of Blount and Patterson, of homologous processes in the Pigeon.

never exhausted during this process of overgrowth. This is due to the fact that as fast as its inner margin becomes converted into cells, new germ wall is created by the peripheral movement of more periblast nuclei into the new periblastic region which lies continually further out. Meanwhile, as the cellular area is thus extended, the original segmentation cavity likewise enlarges beneath it. This augmented central space is then often referred to as the *subgerminal cavity*,[3] whose outward extension as such ceases about the time gastrulation is completed.

The Zone of Junction and the Zone of Overgrowth. — Beyond the extent of the subgerminal cavity, however, the cellular area continues to spread over the yolk. Although the actual cavity as such ceases to expand subsequent to gastrulation, this outgrowth of the cellular region is accompanied by an ever-widening zone, in which the newly formed cells are nevertheless distinctly separated from the underlying yolk. The separation is then continuous at its inner margin with the subgerminal cavity. It should further be noted that at its outer edge this zone of separation extends somewhat beyond the region where the germ wall has been entirely organized, within its deeper portions, into cells. In other words at the inner margin of the germ wall, the latter is already slightly separated from the yolk beneath it (Fig. 138, *D, x*). In its more peripheral part, on the other hand, the germ wall, as already indicated, is quite continuous with the underlying yolk. Likewise, the cells which, even in this outer zone, now cover the upper surface of the wall as fast as it forms, are unseparated by cytoplasmic cleavage from the unsegmented portion of the wall beneath them. Because of this lack of separation, this outer portion of the germ wall is known as the *zone of junction* (Fig. 138, *D*). Lastly, beyond the extreme limit of the zone of junction there exists a narrow superficial rim of cells which extends out over the unsegmented yolk (periblast), from which it is quite separate. This is called the *zone of overgrowth,* and, although arising from the outer edge of the zone of junction, it seems to be maintained by the multiplication of its own cells (Figs. 138, *D,* and 140).

The Blastoderm. — It may now be added that with the appearance of these zones the egg has become a *blastula,* while the entire cellular and partially cellular area, including the zone of junction and the zone of overgrowth, may henceforth be referred to as the *blastoderm* (Fig.

[3] The above distinction between segmentation cavity and subgerminal cavity is frequently not adhered to, the two terms being considered synonymous.

138, D). Its establishment terminates the period of segmentation as distinguished from that of gastrulation. Nevertheless, the outward extension of the blastoderm over the yolk continues for some time after the latter process is completed. This is brought about by the steady out-pushing of the zone of overgrowth and the germ wall, which not only themselves increase somewhat in width (particularly the germ
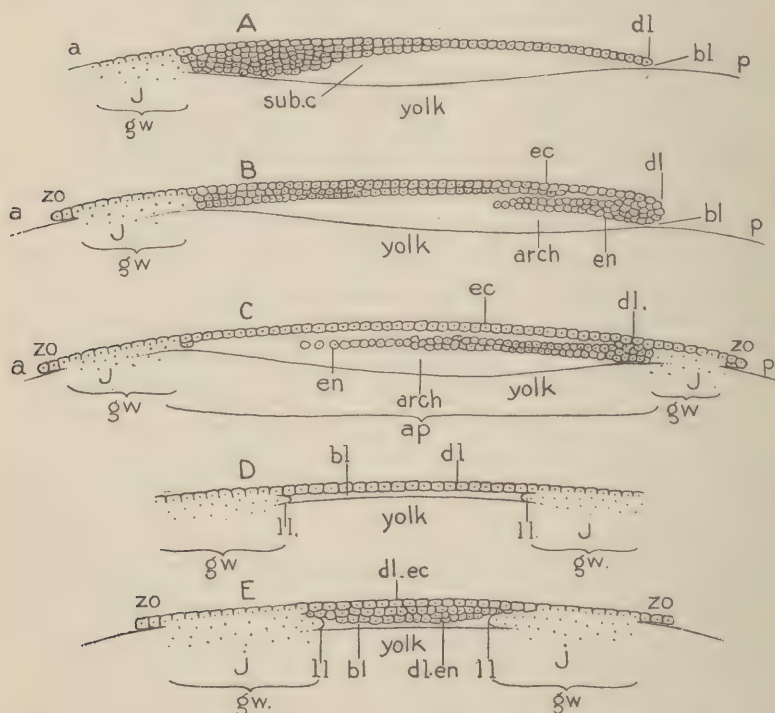
Fig. 139I. — Diagrams of verticle sections through the blastoderm of the Pigeon's egg during the stages of gastrulation indicated in Fig. 140 and based upon figures by Patterson. A. A longitudinal section (i.e. along the line CD) through a blastoderm similar to A in Fig. 140. In this case no zone of overgrowth is yet present. B. A similar section through B, of Fig. 140. C. A similar section through C of Fig. 140. D. An imaginary section across the posterior portion of a blastoderm approximately like A in Fig. 140. In this section the observer is, as it were, looking at the posterior edge of the blastoderm. E. A similar section through a blastoderm such as B in Fig. 140.

a. Anterior. ap. Approximate extent of the area pellucida at the end of gastrulation. $arch$. Archenteron. bl. Blastopore. dl. Dorsal lip. $dl.ec$. Dorsal lip ectoderm. $dl.en$. Dorsal lip endoderm. ec. Ectoderm. en. Endoderm. gw. Germ wall. j. Zone of junction. ll. Lateral lip. p. Posterior. $sub.c$. Subgerminal cavity. zo. Zone of overgrowth.

wall), but leave behind them an ever-widening area of extra embryonic ectoderm, mesoderm, and endoderm. The exact method by which these cell layers are differentiated within the extra-embryonic blastoderm will be discussed in detail later. (Figs. 144 and 149.)

It is worth indicating at this point, however, that the process thus initiated is destined eventually to envelop the yolk in a sac, by a method similar to that with which the student is already familiar in both the Teleosts and Gymnophiona. The fact also that this envelopment is not completed until long after gastrulation has ended, is a feature which should be recalled as particularly comparable with the condition in the latter group of animals. Finally, it is now possible to suggest one further comparison which is highly important. It is fairly obvious that the edge of the blastoderm in the Bird is quite comparable with that in both the Fish and Gymnophiona; i.e., it really represents, at the start at least, the total lip of the blastopore. Then upon the basis of the behavior of this lip in the Gymnophiona, as just noted, the meaning of the features characteristic of avian gastrulation become relatively easy to understand. This topic will be discussed further following a description of this process in the Chick.

GASTRULATION [4]

It has been noted that gastrulation is often begun while the egg is in the lower end of the uterus, and if it be retained there overnight this process may be completed before laying. In any event the steps involved are as follows:

The Area Pellucida and Area Opaca. — The first sign that gastrulation has begun is a thinning of the blastodermal roof. This is due to the rearrangement of the cells so that they once more constitute a single layer; it results apparently from the very rapid expansion of the blastoderm taking place at this time. The thinning process first becomes noticeable near what later proves to be the posterior margin, and spreads anteriorly and laterally (Figs. 139I, A, and 140, A). Eventually (at the end of gastrulation), it reaches slightly beyond the position then occupied by the inner rim of the germ wall; that is to say, approximately to the outer edge of the subgerminal cavity (Figs. 139I, C and 140, C). The region thus affected is known as the *area pellucida;* it constitutes the strictly embryonic portion of the

[4] The account of gastrulation also is from Patterson's description of the process in the Pigeon.

blastoderm.   Beyond this area there is then the blastodermal rim
composed of the outer margin of the germ wall; i.e., the zone of
junction and the zone of overgrowth.   Because of its contrast with
the central region when viewed from the surface, this rim is known
as the *area opaca*, and its extent continues to be virtually coincident
with that of the blastoderm beyond the approximate boundary of the
subgerminal cavity.   The parts to which it gives rise are chiefly
extra-embryonic.

The Formation of the Blastopore and its Lips. — As the roof
at the posterior margin of the blastoderm begins to become thin, there
develops just beneath this margin a gap in the zone of junction and
the germ wall.   Thus at this point, the upper blastodermal layer rests
directly upon the yolk.   The gap may now be said to represent the
blastopore, and the margin of the blastoderm directly over it is its
dorsal lip.   The edges of the germ wall and the zone of junction
forming the sides of the gap are then, for the time being, its *lateral
lips,* while at this stage no true ventral lip exists (Figs. 139I, *A, D,*
and 140, *A*).

Formation of the Embryonic Endoderm and the Archenteron.
— That portion of the blastodermal margin which forms the dorsal
lip now begins to turn under, or become involuted.   The lower layer
thus formed then extends forward through the gap in the germ wall
and spreads out within the subgerminal cavity under the region of
the area pellucida (Figs. 139I, *B, E,* and 140, *B*).   This layer is *endo-
derm,* and the upper layer or blastodermal roof from which it is being
derived may now be termed *ectoderm.*   For the present, the margins
of the endoderm are bounded approximately by the inner rim of the
area opaca; indeed, for the time being, the anterior portion of this
layer is formed only of scattered cells, and does not reach quite to
the anterior extremity of the subgerminal cavity.   The portion of this
cavity between the newly forming endoderm and the yolk below it
is the *archenteron* (Figs, 139I, *C* and 140, *C*).   It thus appears that,
aside from the probable occurrence of concrescence discussed below,
the only gastrulation process involved in the case of the Bird is in-
volution.   As usual the embryo is now called a *gastrula.*

The Closure of the Blastopore. — The gap in the germ wall and
the zone of junction; i.e., the blastopore, is now closed by the coming
together of its edges, the above named lateral lips.   This occurs in
such a manner, however, that the original margin of the blastoderm,

the dorsal lip, is enclosed inside of the reconstituted zone of junction and germ wall. That is to say, this lip lies just at the posterior border of the area pellucida. Lastly, it is now possible to speak of the zone of junction and the germ wall behind this point as the ventral lip, so far as there is one (Figs. 139I, *C* and 140, *C*).
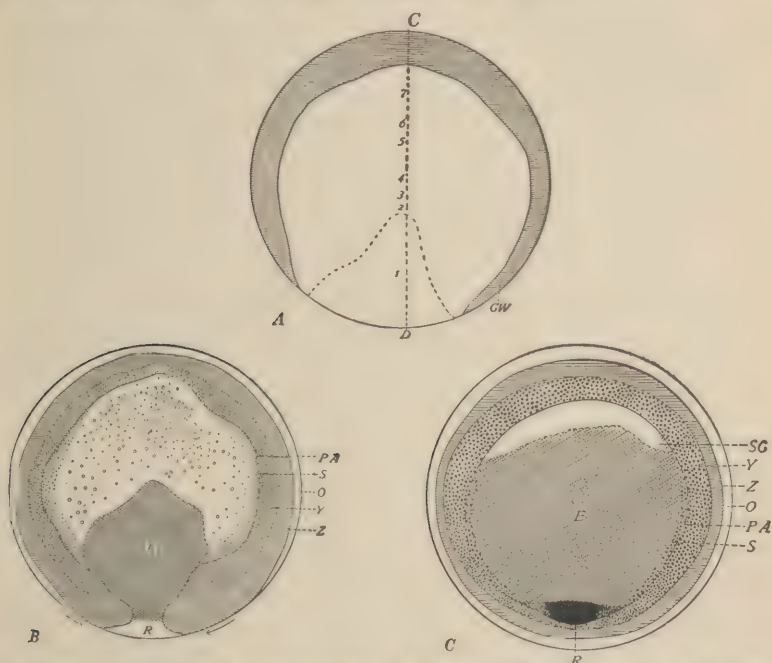
Fig. 140. — Diagrams of reconstructions of the Pigeon's blastoderm. From Lillie (Development of the Chick). After Patterson. Compare figures 138 and 139I. A. Thirty-one hours after fertilization. B. Thirty-six hours after fertilization. C. Thirty-eight hours after fertilization.

E. Gut endoderm. *GW*. Germ wall. *O*. Margin of overgrowth. *PA*. Outer margin of area pellucida. *R*. In B margin of involution, in C mass of cells left after closure of the blastopore. *S*. Beginning of yolk-sac endoderm. *SG*. Anterior part of sub-germinal cavity (blastocœl), as yet free from endoderm. *Y*. Inner portion of germ wall becoming separated from yolk (region X in Fig. 138, D). *Z*. Zone of junction. The numbers 1–7 along the line C–D in A, indicate the number of cells in the thickness of the blastoderm in these regions.

Whether there is any real concrescence involved in this process has been a difficult problem to solve, but on the basis of facts submitted in the following chapter it now appears altogether likely that such is the case. In that event, it follows of course that upon closure, part of the actual lateral lips consist of the margins of the blastoderm which lay immediately adjacent to the original dorsal lip (Fig. 143).

The diameter of the entire blastoderm in the Hen is now approximately 4.41 mm. and that of the area pellucida 2.51 mm. (Edwards). At about this stage the egg is generally laid, and if it is unincubated may remain in this condition for some time. If incubation ensues before too long an interval has elapsed, further development then proceeds as follows.[5]

[5] From this point on the data is the result of investigations of the Hen, not of the Pigeon.

THE CHICK: DEVELOPMENT DURING THE FIRST DAY[1] OF INCUBATION

THE PRIMITIVE STREAK AND RELATED STRUCTURES

THE PRIMITIVE STREAK

Shortly after incubation begins, the endoderm extends throughout the entire region of the area pellucida, so that the latter consists everywhere of two layers, ectoderm and endoderm. A narrow opaque band now appears in the area pellucida extending from a point slightly anterior to the middle of it almost to its posterior margin. This band is caused by a thickening of the ectoderm and is known as the *primitive streak*. The slight space between its end and the posterior edge of the area pellucida is called the *primitive plate* (Fig. 141, *A*). As the streak elongates posteriorly, the primitive plate persists between it and the edge of the area pellucida, so that the posterior region of the latter is necessarily lengthened. Thus the whole area becomes pear shaped, with the larger end anterior. This change in shape of the area pellucida is a noticeable feature at this stage. A groove presently develops down the middle of this streak and terminates at the anterior end in a slight pit. These structures are known respectively as the *primitive groove* and the *primitive pit*, while the slightly raised ridges which bound the groove on either side are called the *primitive folds*.[2] Lastly the fusion of the latter in front of the primitive pit is termed the *primitive knot* or *Hensen's knot*. Fig. 142, *B*, *C*, represents transverse sections through the head process and the primitive knot respectively, which show the relations of these structures to the ectoderm and endoderm.

[1] The term day as used in connection with the development of the Chick indicates a period of twenty-four hours.

[2] The term primitive streak is often used to refer to the entire structure.
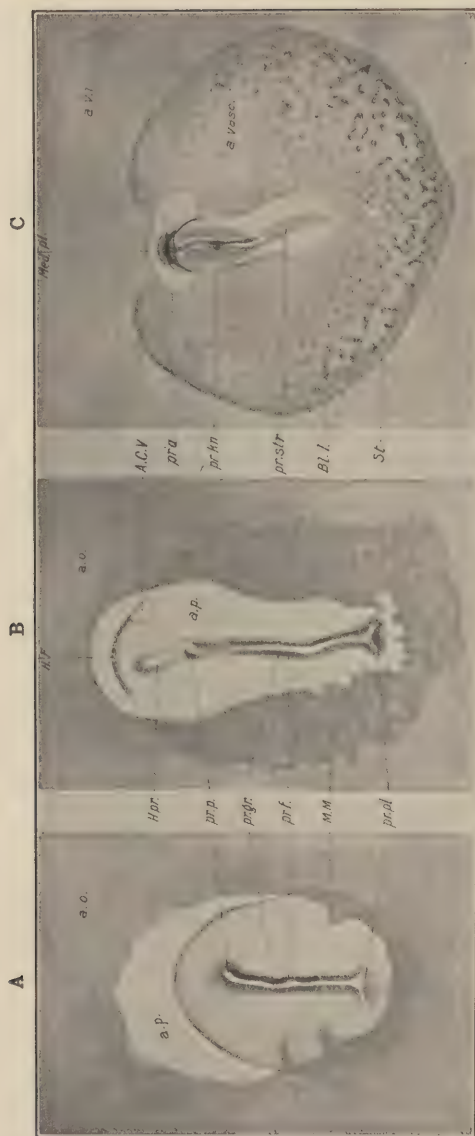
Fig. 141. — Three stages of the blastoderm to show the primitive streak, head process, extension of the mesoblast, and related developments. From Lillie (Development of the Chick).

A. Before formation of the head-process.

B. The head-process is formed; the head fold in process of formation.

C. Later stage shortly before the appearance of the first intersomitic groove.

A.C.V. Amnio-cardiac vesicle. *a.o.* Area opaca. *a.p.* Area pellucida. *a.vasc.* Area vasculosa. *a.v.i.* Area vitellina interna. *Bl.I.* Blood island. *H.F.* Head-fold. *H.pr.* Head-process. *Med.pl.* Anterior end of medullary plate. *M.M.* Margin of the mesoblast. *pr′a.* Proamnion. *pr.f.* Primitive fold. *pr.gr.* Primitive groove. *pr.kn.* Primitive knot. *pr.p.* Primitive pit. *pr.pl.* Primitive plate. *pr.str.* Primitive streak. *St.* Sinus terminalis.

The Head Process

Its Appearance. — Beginning at its anterior end, the primitive
streak now starts to shorten; concurrently, there is a backward move-
ment of the primitive knot and pit. These features are thus always
found at the anterior extremity of the streak, no matter how much
the latter is shortened. As the primitive streak recedes there appears
in front of it, and lying in the same axis, another darkish line not
unlike the streak in appearance. It is fainter, however, and without
any groove. The posterior end of this line connects with the anterior
end of the streak, and as the latter shortens the new line lengthens so
as to remain in contact with Hensen's knot. This new line is the
head process (Fig. 141, *B, C*).

Method of Origin and Identity with the Notochord. — The exact
method by which this head process originates is somewhat in doubt,
but it seems likely that it is simply the original anterior end of the
streak. It has become differentiated from the streak by the separation
of the superficial layer of ectoderm from the thickened rod beneath
(Fig. 142, *A*). Thus as this separation progresses posteriorly, the
rod, or head process, grows at the expense of the primitive streak.
Whatever its origin, it represents the future *notochord*. It is to be
noted, however, that unlike the notochords thus far studied, this
one apparently arises from the ectoderm rather than the endoderm,
though it becomes attached to the latter for a time.

The Origin of the Embryonic Mesoderm

As the above changes take place, cells are proliferated both from the
sides of the primitive streak and from the head process. At first
these are scattered, but presently they begin to form a sheet, which
extends out between the ectoderm and endoderm; it is the *mesoderm*.

The Nature of the Primitive Streak

The question as to whether the primitive streak of the Bird can be
homologized with the blastopore and its lips in the Fish and Amphibian
has long been a problem confronting embryologists. It is now held
by many that such a homology does exist. Before citing the reasons
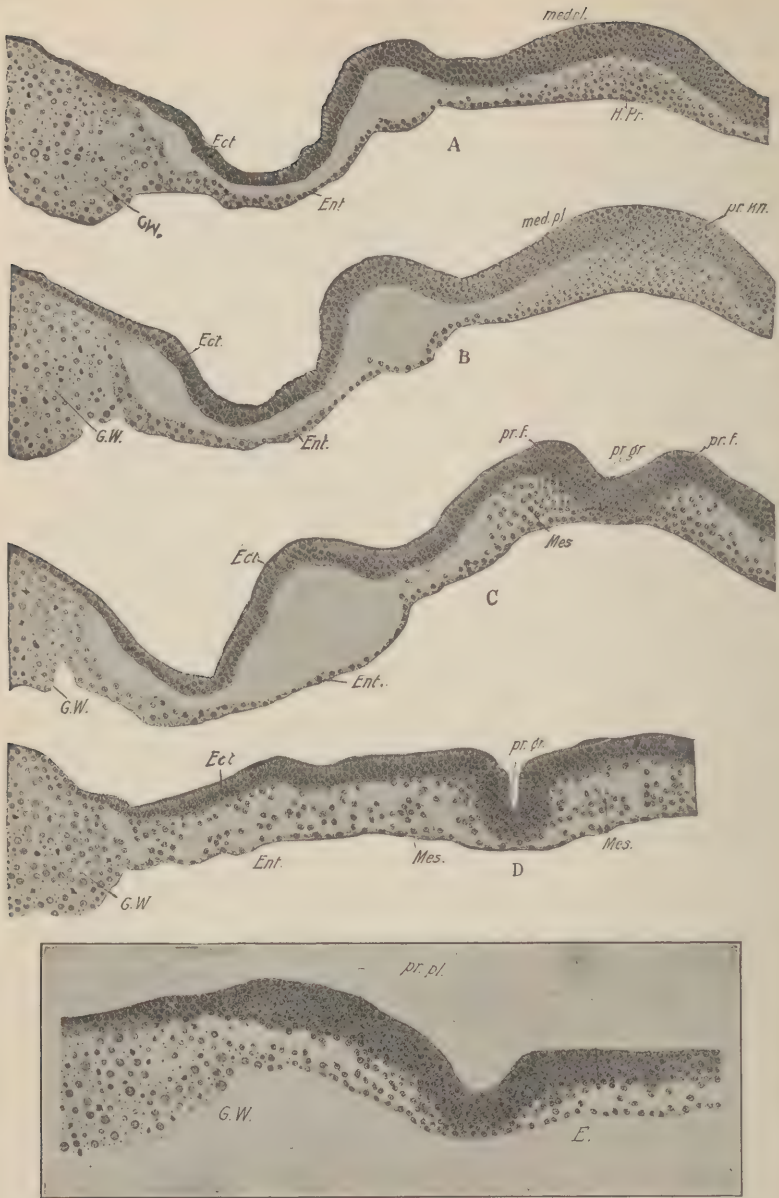
Fig. 142. — Five transverse sections through the head process and primitive streak of a Chick embryo. The head process is very short. From Lillie (Development of the Chick).

A. Through the head process, now fused to the entoderm. B. Through the primitive knot. C. Through the anterior end of the primitive groove. D. A little behind the center of the primitive streak. E. Through the primitive plate. The total number of sections through the head process and primitive streak of this series is 102. B. is 4 sections behind A. C is 12 sections behind A. D is 59 sections behind A. E is 87 sections behind A.

Ect. Ectoderm. *Ent.* Entoderm. *G.W.* Germ wall. *H.Pr.* Head process. *med.pl.* Medullary plate. *Mes.* Mesoblast. *pr.f.* Primitive fold. *pr.gr.* Primitive groove. *pr.kn.* Primitive knot. *pr.pl.* Primitive plate.

for this belief, however, it may be well to call attention to the three chief respects in which the primitive streak of Birds differs from the structures with which it is sought to homologize it.

The Differences Between the Primitive Streak and a Typical Blastopore.

(1) The blastopore of the Bird, developed during gastrulation by means of involution, as described above, has no direct connection with the primitive streak; i.e., the latter is not actually seen to arise out of the former by a process of concrescence, as was the case with the Frog, the Fish, and the Gymnophiona. Instead, the primitive streak arises sometime after the blastopore and its lips have disappeared.

(2) The primitive groove, which would be the part of the streak really equivalent to the blastopore itself, does not at any time actually communicate with the archenteron, as does the blastopore in the Frog or the Fish.

(3) Finally, the primitive folds, which form the sides of the primitive streak and correspond to the lips of the blastopore, do not connect beneath with the endoderm as blastoporal lips typically do.

In spite of these differences, however, the following rather striking facts have led many embryologists to a belief in the homology indicated above.

Reasons for Identifying the Blastopore and Primitive Streak

(1) In general, the embryo is differentiated in front of the primitive streak in a Bird, just as it is differentiated in front of the primitive streak, or blastopore, of the Frog or Fish.

(2) The notochord in Birds, besides being immediately anterior to the primitive streak; i.e., the " blastoporal lips," is differentiated out of material derived from it. It has already been indicated, however, that the lips of the blastopore in animals like Amphioxus and the Frog are also the primary source of most of the notochordal material.

(3) A large part of the mesoderm in the Bird takes its origin from the lateral edges or lips of the primitive streak in virtually the same way that a part of the mesoderm; i.e., the peristomial mesoderm, arises from the lateral lips of the blastopore in Amphioxus, the Frog, and Fish. Thus, as in these animals we have gastral and peristomial mesoderm, so in the Bird we may refer to the more anterior mesoderm pro-

liferated by the head process as axial or gastral, and the mesoderm proliferated by the primitive streak as peristomial.

(4) In some Birds (Duck, Goose and others) the primitive pit at the anterior end of the primitive streak is covered by the medullary
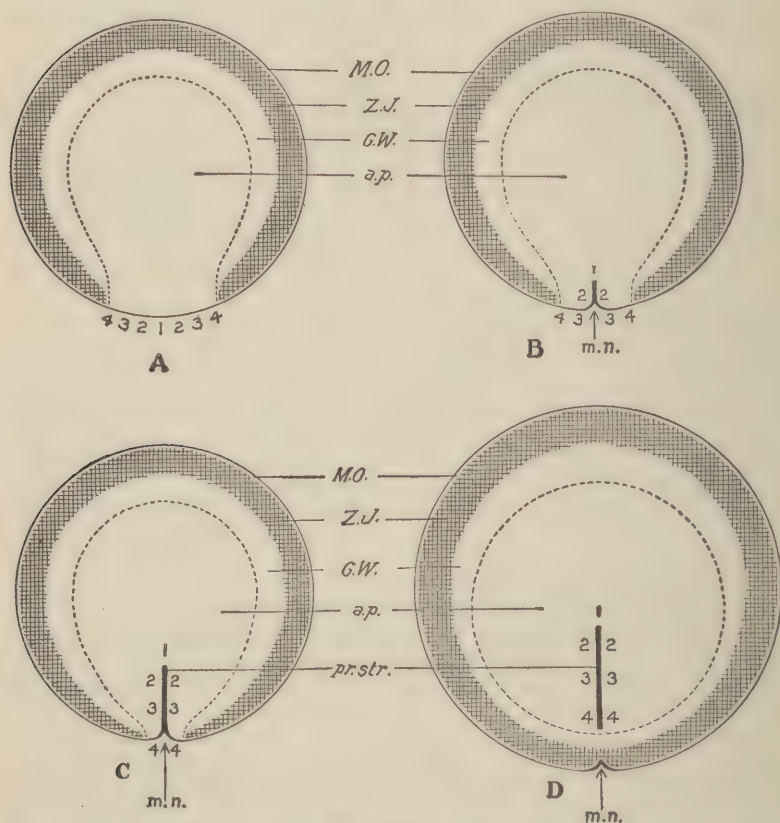
Fig. 143. — Diagrams to illustrate the theory of concrescence as applied to the primitive streak of the Bird. From Lillie (Development of the Chick). The central area bounded by the broken line represents the pellucid area; external to this is the area opaca, showing as concentric zones the germ-wall (*G.W.*), the zone of junction (*Z.J.*), and the margin of overgrowth (*M.O.*).
a.p. Area pellucida.   *m.n.* Marginal notch.   *pr. str.* Primitive streak.

folds and eventually opens into the enteron. Thus there is formed a typical neurenteric canal in just the region where such a canal would be expected to appear, were the primitive groove a blastopore.

(5) The anus forms at the posterior end of the primitive streak

in the Bird, just as it forms at the posterior end of the blastopore in the Frog.

(6) Perhaps the most significant evidence of all, however, as regards the relationship of the primitive streak and the blastopore, is that obtained in an effort to demonstrate concrescence. Thus Patterson, in his study of gastrulation in the Pigeon, inflicted small injuries upon the margins of the blastoderm as much as 45° to either side of the blastopore during the period of its closure. He then found that these injuries appeared later in the primitive streak, from the material of which the axial rudiments of the embryo were then differentiated as described above. These results would, therefore, seem to demonstrate, first, that the injuries must have been carried into their final positions by a process of concrescence, and second, that the primitive streak really represents the closed blastopore. This, it is true, has been shown only in the case of the Pigeon, but in all probability it is true of other Birds as well (Fig. 143).

Comparison with the Gymnophiona. — Finally, in concluding this discussion, the whole situation will be rendered more clear if the student will compare the condition described above with that which exists in the Gymnophiona. In that case, it will be recalled, the anterior and lateral parts of the blastoderm do not grow around the yolk until long after the blastopore is closed. The closure of the latter occurs, therefore, simply by a swinging together of the lateral lips, and their posterior fusion to form a ventral lip. If, now, the outer rim of the blastoderm in the Gymnophiona be homologized with the outer rim in the Birds; i.e., with the region of the germ wall and the zone of junction, it will be seen that almost exactly the same process takes place. The main difference is that in the Gymnophiona the connection between closed blastopore and " primitive streak " is not obscured by a brief period during which the latter is not visible. Furthermore, in these animals there occurs no break between ectoderm and endoderm at the blastoporal lips, as is apparently the case in the primitive streak of Birds. Finally, as to the matter of concrescence, it will be remembered that by virtue of the above method of closure this process in the Gymnophiona was said to be relatively limited as compared with its extent in an animal like the Frog. It now appears that the same thing holds true in respect to the Pigeon and probably the Chick, only about ninety degrees of the blastodermal margin being involved.

EXTENSION OF THE GERM LAYERS AND FORMA-
TION OF THE AREAS VASCULOSA AND VITELLINA

Mesoderm in the Area Pellucida

Upon either side of the head process and primitive streak, the meso-
derm extends outward in a lateral and posterior direction until it
reaches the boundary of the area pellucida. This growth is achieved
by simple cell proliferation at the edges of the mesodermal sheet, and
hence some claim that the origin of this layer in the area opaca is due
merely to a continuation of this process. It seems more likely, how-
ever, that the extension into this latter area is at least partly accom-
plished in another manner, as follows:

Blood Islands and the Mesoderm and Entoderm in the
Area Opaca

Somewhat before the presence of mesoderm can be demonstrated
in the area opaca, there appear around its postero-lateral border upon
the surface of the germ wall beneath the layer of ectoderm, small
masses of cells containing yolk granules. These masses presently
anastamose with one another to form a network, while throughout this
network lacunæ are developed containing groups of cells. Thus the
connecting lacunæ come to constitute blood vessels, while the cells
within them become transformed into blood corpuscles. Because of
these facts, therefore, the original cell masses are termed *blood islands*
(Fig. 144, *C*). As the blood islands are becoming transformed into
vessels, however, they are said to bud off certain cells between them-
selves and the ectoderm. It is these cells, together with others ap-
parently derived from the edges of the mesoderm already existing,
which are thought to form a large part of the mesoderm of the area
opaca. At all events, as soon as it is defined, the mesoderm of this
area seems to become entirely continuous with that of the pellucid
area at its inner border. After the formation of the blood islands and
mesoderm from the upper layer of the germ wall, the deepest layer
of the wall beneath the newly formed blood vessels becomes rapidly
differentiated into the *yolk-sac endoderm*. It is continuous at the
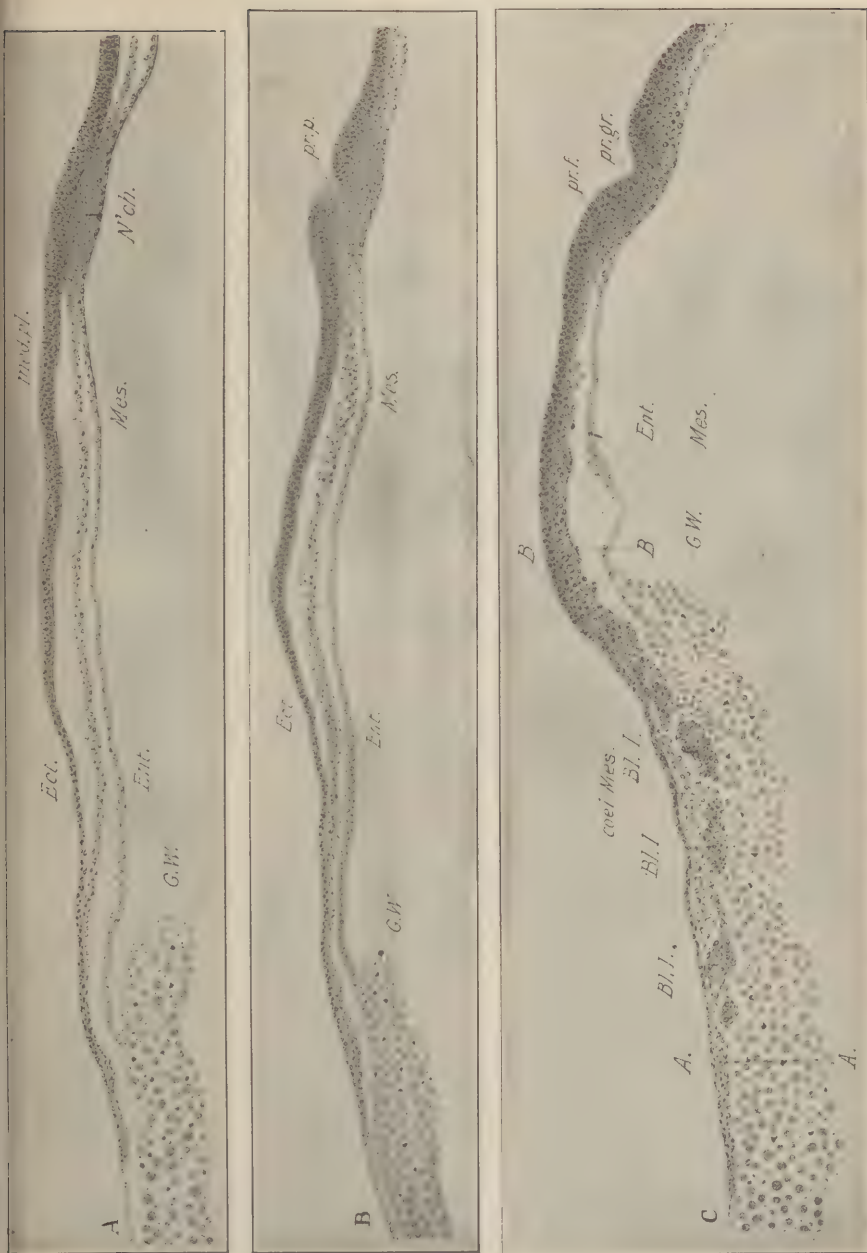
Fig. 144.—Three transverse sections of a late stage (corresponding to about Fig. 141, B), through the head-process and primitive streak of a Chick embryo. From Lillie (Development of the Chick). A. Near the hind end of the head-process. B. Through the primitive pit. C. A short distance behind the center of the primitive streak. *Bl.I.* Blood island. *coel.Mes.* Cœlomic mesoblast. *Ect.* Ectoderm. *Ent.* Entoderm. *G.W.* Germ-wall. *med pl.* Medullary plate. *Mes.* Mesoderm in area pellucida. *N'ch.* Notochord. *pr.f.* Primitive fold. *pr.gr.* Primitive groove. *pr.p.* Primitive pit.

inner border of the area vasculosa with the embryonic endoderm produced within the area pellucida by the proliferation of the endodermal cells involuted at the lip of the blastopore (Fig. 144). From this description, therefore, it appears, as previously suggested, that though the origin of the extra-embryonic endoderm occurs in connection with a general epiboly of the blastoderm, its immediate production involves a process of virtual delamination.

Meanwhile, the processes thus described are gradually working forward upon each side of the area opaca, the proliferated mesoderm of the area pellucida keeping pace with that which arises from the blood islands further out. Finally, as the level of the anterior end of the head process is reached, the mesoderm of the pellucid area ceases to form. That in the area opaca, however, continues upon either side as a pair of anteriorly projecting wings, which after proceeding somewhat beyond the future head region begin to turn toward one another so that they eventually meet (see second day). In the area pellucida, however, immediately in front of and slightly to the sides of the head region, no mesoderm forms for some time, the zone thus marked out being termed the *proamnion* (Fig. 141, *C*).

The Area Vasculosa. — It must now be noted that the blood vessels, having once become formed in the area opaca, are not confined there. Very soon, especially postero-laterally, they begin to extend into the area pellucida, where they unite with other vessels which have arisen *in situ* from the mesoderm; the entire region thus covered by them is then termed the *area vasculosa*. Presently around the outer edge of this area, its boundary begins to be clearly defined by an encircling blood vessel, the *sinus terminalis* (Fig. 141, *C*).

The Area Vitellina. — Finally, beyond the sinus terminalis the remainder of the blastoderm, consisting of the regions of the germ wall and the zone of overgrowth, is known as the *area vitellina*. This area is then further subdivided into an inner and an outer portion as follows: The outer portion consists of the zone of overgrowth and that part of the germ wall comprising the zone of junction; it is known as the area vitellina *externa*. Between this area and the sinus terminalis there then occurs a broader region of the germ wall in which the deeper cytoplasm is beginning to show that definite differentiation into cells which is already evident everywhere upon its surface; it is the area vitellina *interna*. This area is particularly distinguished from that further out, moreover, by the fact

that in it the germ wall is slightly separated from the unnucleated yolk beneath it, the separation being continuous centrally with the margins of the subgerminal cavity. In other words within this inner area the germ wall no longer constitutes a true zone of junction between the blastoderm and the yolk. Upon the other hand, though covered superficially by a layer of cells called ectoderm, such other cells as occur within the deeper parts of the wall of this area are not yet differentiated
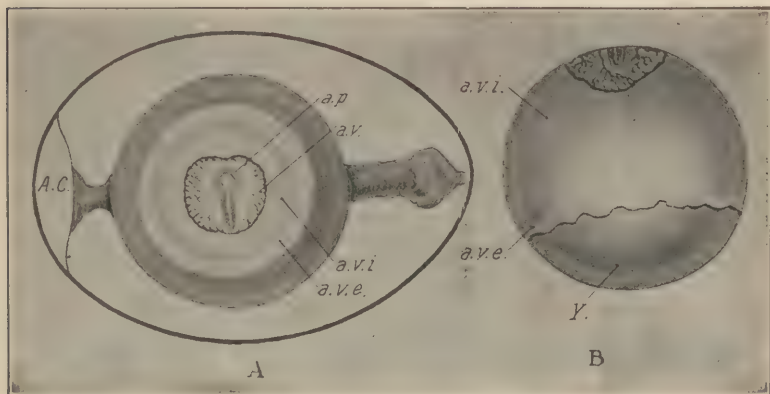
Fig. 145. — A. Hen's egg at about the twenty-sixth hour of incubation. to show the zones of the blastoderm and the orientation of the embryo with reference to the axis of the shell. B. Yolk of hen's egg incubated about 50 hours to show the extent of overgrowth of the blastoderm. From Lillie (Development of the Chick). After Duval.

A.C. Air chamber. a.p. Area pellucida. a.v. Area vasculosa. a.v.e. Area vitellina externa. a.v.i. Area vitellina interna. Y. Uncovered portion of yolk; i.e., the "yolk blastopore" or yolk-sac umbilicus (see below, page 338).

into definite mesoderm and endoderm as is the case within the area vasculosa (Fig. 149).

As has already been suggested, all of these areas, while retaining the same relative position as regards each other, are constantly moving outward over the surface of the yolk (Fig. 145).

THE HEAD FOLD AND FORE–GUT

THE HEAD FOLD

A short distance in front of the anterior end of the head process, there develops shortly a slight depression, and immediately posterior

Fig. 146. — Median sagittal section. Stage of the first intersomitic groove. (Cf. Fig. 147.) Owing to the bending of the primitive streak the section passes to one side of the middle line posteriorly. From Lillie (Development of the Chick).
Ect. Ectoderm. *F.G.* Fore-gut. *G.W.* Germ-wall. *H.F.* Head-fold. *med.pl.* Anterior end of medullary plate. *Mes.* Mesoderm. *N'ch +
Ent.* Notochord and entoderm. *Pr'a.* Proamnion. *pr.kn.* Primitive knot. *pr.p.* Primitive pit. *pr.str.* Primitive streak. *Y.S.Ent.* Yolk-sac
entoderm.

to this depression a crescentic fold appears, involving both ectoderm and endoderm (Figs. 146 and 147). Its ends extend almost from one side of the area pellucida to the other. The crest of this fold is not raised perpendicularly to the surface, but extends forward so that it overhangs the depression indicated above. It is the *head fold,* and its anterior edge marks the anterior end of the embryo. The lateral and posterior limits of the embryo are not distinguishable until much later.

THE FORE-GUT

From the method of its formation, the cavity within the head fold is necessarily lined by endoderm which is co-extensive with the endoderm of the archenteric cavity posterior to it. It is the anterior portion of the future *fore-gut,* the portion which may be said to represent the pharyngeal region. It is a broad, flattened cavity, and as suggested, opens posteriorly into the extensive archenteric space over-lying the yolk. The region of this wide opening is known as the *anterior intestinal portal.* The endoderm on the antero-ventral side of the fore-gut soon fuses with the ectoderm below it in a limited region to form the *oral plate* (Fig. 148); elsewhere between the ectoderm and endoderm of this vicinity, there are scattered mesoderm cells; i.e., mesenchyme.

DIFFERENTIATION OF THE EMBRYONIC MESO-
DERM IN THE AREA PELLUCIDA

THE SOMITES AND LATERAL PLATES

The lateral sheets of mesoderm of the area pellucida now become thickened along either side of the head process and primitive streak. The ridges thus formed are known as the *vertebral* or *segmental plates*, while the remaining lateral portions of the sheets are called the
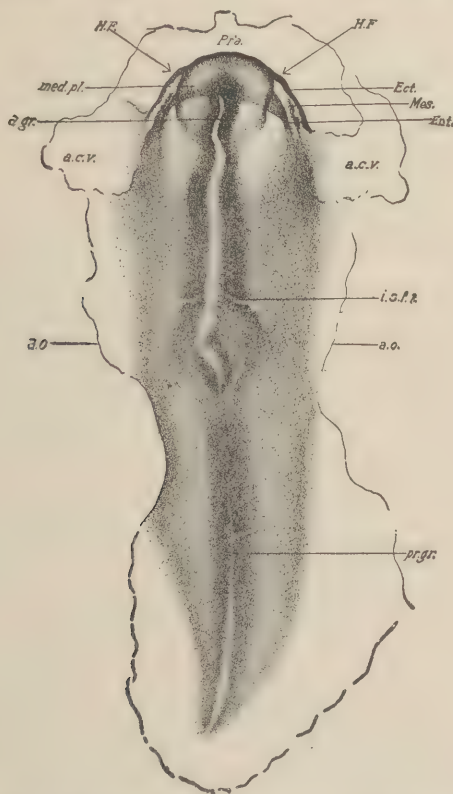
Fig. 147. — Stage of first intersomitic groove drawn from an entire mount in balsam by transmitted light. From Lillie (Development of the Chick).

a.c.v. Amnio-cardiac vesicle. *a.o.* Inner margin of Area opaca. *Ect.* Ectoderm. *Ent.* Entoderm. *H.F.* Head-fold. *i.s.f.1.* First intersomitic furrow. *med.pl.* Anterior end of medullary plate. *Mes.* Mesoderm. *n.gr.* Neural groove. *pr.gr.* Primitive groove. *Pr'a.* Proamnion.

lateral plates. Just in front of the anterior end of the primitive
streak a transverse fissure now appears in each of the vertebral plates.
The region of the plates immediately anterior to these fissures then
constitutes the *first pair* of *somites;* they remain continuous anteriorly
with the mesoderm of the head region (Fig. 147). A short distance
behind the first pair of fissures a second pair presently develops, and
the portion of the vertebral plates between the first and second pairs
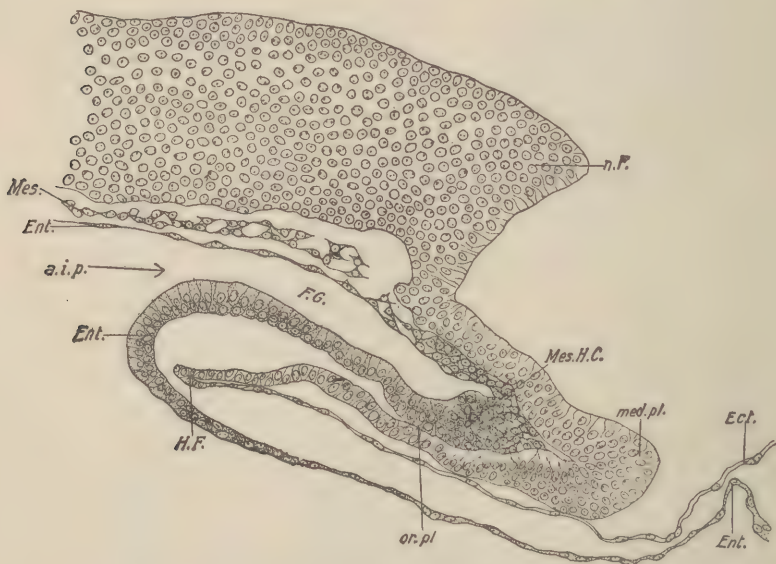
Fig. 148. — Median longitudinal section of the head, stage of 4 s. The section passes
through the length of one of the neural folds just behind the anterior end. From Lillie
(Development of the Chick).

a.i.p. Anterior intestinal portal. *Ect.* Ectoderm. *Ent.* Entoderm. *F.G.* Fore-gut.
H.F. Head-fold. *Mes.* Mesoderm. *Mes.H.C.* Mesoblastic head cavity. *n.F.* Neural
fold. *or.pl.* Oral plate.

of fissures is the second pair of somites. This process of segmentation
then continues until by the end of twenty-four hours about five more
pairs of somites have thus been formed, all posterior to the first pair
and in front of the primitive streak, which is correspondingly shortened.
This means, of course, that they lie upon either side of the head process,
which, as has already been indicated, is really the rudiment of the
notochord. The first four pairs of these somites later disappear, being
included in the posterior part of the head.

Fig. 149. — A. Transverse section across the axis of the embryo and the entire blastoderm of one side. The section passes through the sixth somite of a 10s embryo, and is intended to show the topography of the blastoderm. The regions B, C, D, E are represented under higher magnification in the Figs. B, C, D, E. From Lillie (Development of the Chick).

Ao. Dorsal aorta. a.v.e. Area vitellina externa. a.v.i. Area vitelline interna. Bl.i. Blood island. Bl.v. Blood vessel. Coel. Cœlom. G.W. Germ wall. M.O. Margin of overgrowth. Nch. Notochord. NF. Neural fold. Nph. Nephrotome. S. Somite. Somp. Somatopleure. Spl'pl. Splanchnopleure. Som.Mes. Somatic layer of mesoblast. spl.Mes. Splanchnic layer of the mesoblast. S.T. Sinus terminalis. Y.S.Ent. Yolk-sac entoderm. Z.J. Zone of junction.

The Nephrotome. — A narrow strip of each lateral plate immediately adjacent to the somites serves, as it were, to unite them to the main part of the plate. It is known as *the nephrotome,* and later gives rise to the excretory organs.

THE CŒLOM

Within the lateral sheets, which for a time remain connected with the somites by means of the nephrotomes, horizontal splits now develop. They occur first in the anterior portions and gradually spread elsewhere. Of the two sheets thus formed, the one next to the ectoderm is the *somatic* or *parietal mesoderm* (*somatopleure*), and that next to the endoderm the *splanchnic* or *visceral mesoderm* (*splanchnopleure*). The space between them is the *cœlom* (Fig. 149).

THE RUDIMENT OF THE PERICARDIAL CAVITY

In the region of the head fold, the cœlomic spaces on each side push toward each other. By so doing, they finally work their way in between the ectoderm and endoderm just at the bend where these two layers pass up from the depressed area under the fold on to its ventral surface. At the end of twenty-four hours, the walls of the opposite spaces have met each other and fused, so that the spaces themselves are separated only by a thin layer of mesoderm. This process tends to separate the ectoderm and the endoderm by pushing the latter further back, and thus increasing the length of the fore-gut. These in-pushing portions of the cœlom are the *amnio-cardiac vesicles,* and they represent the rudiment of the *pericardial cavity* (Figs. 150, 161).

THE NERVOUS SYSTEM

Among the most conspicuous features of the early embryo is the rudiment of the central nervous system. This system first appears in the following manner:

THE MEDULLARY OR NEURAL PLATE

Beginning almost at the anterior limit of the head fold the ectoderm above and along each side of the head process is thickened somewhat;

this thickening is the *medullary plate*. Posteriorly, the lateral portions of the plate extend also along each side of the primitive streak, while the central portion merges with the ectoderm of the primitive groove.

THE MEDULLARY GROOVE AND MEDULLARY FOLDS

Presently a depression appears running down the middle of the medullary plate above the head process, and on each side of this de-
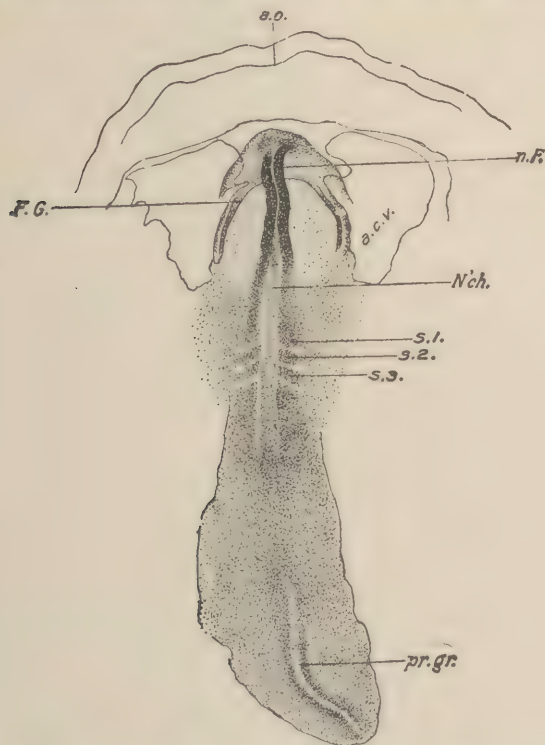
Fig. 150. — Chick embryo with three pairs of somites (about twenty-three hours). Dorsal view. From Lillie (Development of the Chick).

a.c.v. Amnio-cardiac vesicle. *a.o.* Inner margin of area opaca. *F.G.* Fore-gut. *N'ch.* Notochord. *n.F.* Neural fold. *pr.gr.* Primitive groove. s_1, s_2, s_3. First, second and third somites.

pression, the lateral portions of the plate rise up as two parallel ridges. The depression is, of course, the *medullary groove*, while the ridges are the *medullary folds* (Fig. 150). Approximately at the anterior end of

the plate, the ends of the folds meet one another. However, because of the fact that they are already quite close together, this meeting does not form an extensive transverse ridge as in the Frog. Posteriorly, the folds do not at first reach quite to the region of the first somite, but before the end of the day they have extended backward to about the anterior end of the shortened primitive streak.

The Neural Tube

The parallel medullary folds now bend toward one another until their crests meet and fuse a little distance posterior to the anterior limit of the head fold, in the region of the future mid-brain. As in the case of the Frog, a continuation of this fusion results in the formation of a thick-walled tube, whose roof, sides, and floor are derived from the inner walls of the medullary folds and from the groove; it is the *neural tube* and its cavity of course is the *neural canal*. As in the Frog, also, there occurs shortly after the fusion of the folds, a separation between their inner (neural) and outer walls, the latter reconstituting above the tube a continuous layer of ectoderm.

These processes continue both anteriorly and posteriorly until the tube is entirely closed in. During the closure, however, the usual anterior and posterior openings into the neural canal persist. The former is the *neuropore,* corresponding to the structure of that name in the forms previously studied; this opening is closed during the first day. It should also be noted that because of the protrusion of the folds in this region, they extend forward slightly beyond the anterior limit of the fore-gut (Fig. 148). Later, as growth proceeds, this region is actually carried over the anterior end of the embryo on to the ventral side (see below under flexures). Posteriorly fusion takes place more rapidly, keeping pace with the extension of the medullary folds. Because of the greater distance to be traversed, however, the process in this direction is not completed until some time later. The completion at this end is marked by the disappearance of the primitive streak (Fig. 151).

The Neural Crests

At the same time that fusion of the folds is occurring, cells are proliferated between the outer and inner layers of each fold, just in the region of its crest. Thus, as fusion takes place, these cells form a band
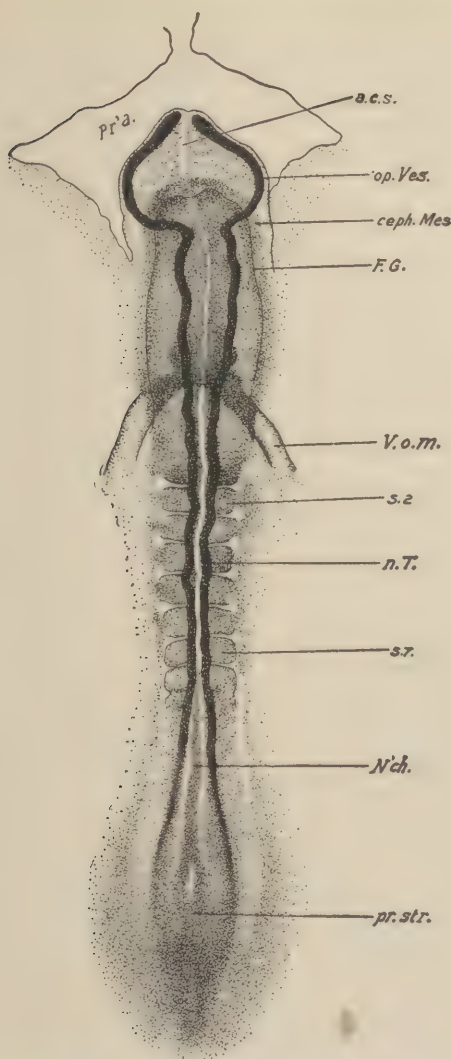
Fig. 151. — Chick embryo with seven pairs of somites (about twenty-five hours)
Dorsal view. From Lillie (Development of the Chick).

a.c.s. Anterior cerebral suture; i.e., line of fusion of neural folds here.   ceph.Mes.
Cephalic mesoderm.   F.G. Fore-gut.   N'ch. Notochord.   n.T. Neural tube.   op.Ves.
Optic vesicle.   Pr'a. Proamnion.   pr.str. Primitive streak.   s.2, s.7. Second and seventh
somites.   V.o.m. Omphalomesenteric (vitelline) vein.
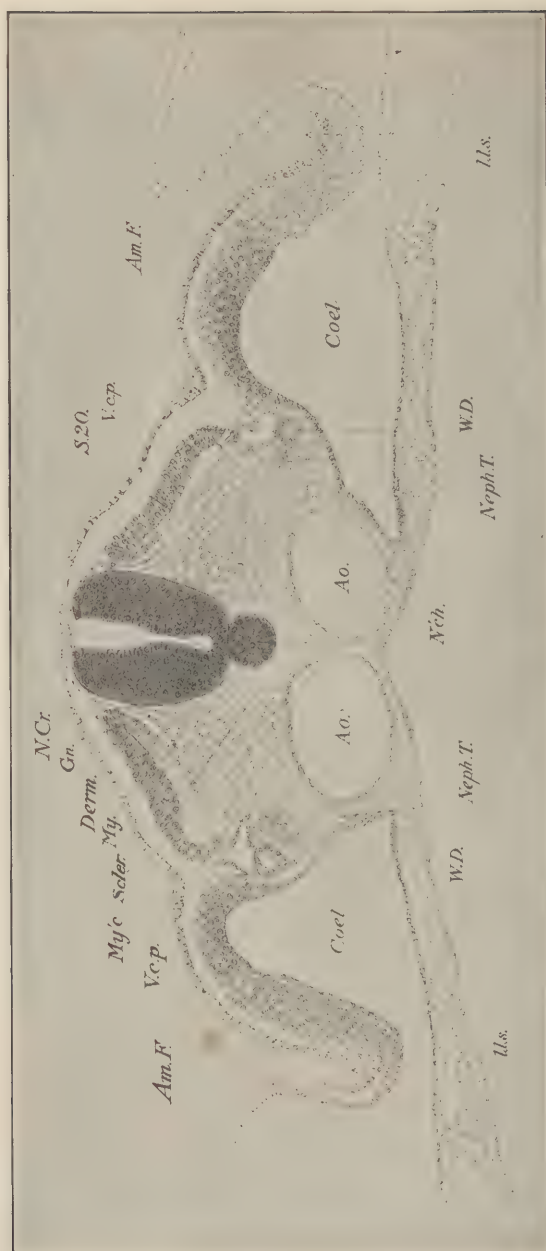
Fig. 152. — Transverse section through the twentieth somite of a 29 s embryo. From Lillie (Development of the Chick). *Am.F.* Amniotic fold. *Ao.* Aorta. *Coel.* Cœlom. *Derm.* Dermatome. *Gn.* Ganglion. *l.l.s.* Lateral limiting sulcus (see page 337). *My.* Myotome. *My'c.* Myocœl. *N'ch.* Notochord. *N.Cr.* Neural crest. *Neph.T.* Nephrogenous tissue. *S.20.* Twentieth somite. *Scler.* Sclerotome. *V.c.p.* Posterior cardinal vein. *W.D.* Wolffian duct.

along either side of the dorsal part of the neural tube between it and the surface ectoderm. These bands are the *neural crests*, which at this time are united with one another across the dorsal surface of the tube (Fig. 152).

THE OPTIC VESICLES

Anterior to the first point of fusion, the neural tube is broadened somewhat. This is the region of the future *optic vesicles*.

SUMMARY OF THE CONDITION AT THE END OF THE FIRST DAY OF INCUBATION

I. THE MESODERMAL STRUCTURES

About six pairs of *somites* are present, lying in front of the primitive knot and connected with the *mesoderm* of the respective *lateral plates* by the longitudinal *nephrotomal* bands.

The lateral mesoderm extends throughout the area pellucida except in the region of the *proamnion*, and together with the endoderm is being differentiated in the area opaca. In the latter area, the formation of this layer has progressed anteriorly until a pair of wing-like extensions are level with the tip of the head fold. Also in the area pellucida this mesoderm has been split into two sheets, the *somatopleure* and *splanchnopleure*, with the *cœlomic* space between them, and this process is spreading into the area opaca. Beneath the fore-gut, the walls of the *amnio-cardiac* portions of the cœlom have just met each other, and the rudiment of the *pericardial cavity* is thus indicated in this region.

In connection with the formation of the mesoderm, *blood vessels* and *corpuscles* have started to appear in the area opaca and area pellucida, transforming both into the *area vasculosa*. The latter is beginning to be bounded by the *sinus terminalis*.

Outside the area vasculosa is an area consisting only of partially differentiated germ wall, the zone of junction, and the zone of overgrowth, the *area vitellina*.

II. THE HEAD FOLD AND THE FORE-GUT

The *head fold* has formed and in the process has given rise to the anterior or pharyngeal portion of the *fore-gut*.

III. The Rudiments of the Nervous System

The *medullary folds* have appeared in the region in front of the primitive knot and have fused for a short space at their anterior ends. The *neural crests* have begun to appear, and the rudiments of the *optic vesicles* are also indicated.

CHAPTER X

THE CHICK: DEVELOPMENT DURING THE SECOND DAY OF INCUBATION

THE SOMITES

When last mentioned the somites were described as masses of meso-derm connected with the lateral plates by means of the nephrotomes. During the second twenty-four hours the connection between nephro-tome and somite is obliterated throughout the greater part of the embryo; the number of pairs of the latter increases to twenty-seven, and beginning at the anterior end the development of each of them proceeds as follows:

THE MYOTOMES AND THE CUTIS PLATES

Each somite is at first composed of an outer layer of comparatively dense cells surrounding an inner mass of mesenchyme, the latter com-parable to a *myocœl*, so far as one exists (Fig. 149, *B*). Presently, however, the denser layer of cells on the side of the somite next to the nerve cord and notochord largely disappears, leaving the latter struc-tures in direct contact with the mesenchymatous mass indicated above. At the same time the dense layer upon the dorsal and outer side of the somite becomes thicker. The dorsal portion of this outer layer is the rudiment of the *myotome*, while the more lateral and ventral portion is the *cutis plate* or *dermatome*. Before the second day has passed, the dorsal or myotomal portion of the above plate of cells begins to turn sharply upon itself and grow downward between the mesenchyme and the cutis plate. Thus a double layer begins to be formed con-sisting of the cutis plate on the outside and the myotomal plate on the inside (Fig. 152).

THE SCLEROTOME

The mesenchyme which now begins gradually to surround the notochord and the ventro-lateral region of the nerve cord is the rudi-ment of the *sclerotome*.

THE ALIMENTARY TRACT

THE FORE-GUT

The Stomodæum. — During the first day it was noted that the antero-ventral end of the fore-gut came in contact with the ectoderm at a point on the ventral side of the head fold to form the oral plate. Now, as the result of the downward flexure of the head and also of the pushing forward of the mandibular arches (see below), the central region of the plate becomes relatively depressed to form a pit lined by ectoderm. It is the beginning of the *stomodæum*, and by a continuation of the above process it presently acquires a considerable depth.

The Hypophysis. — From the antero-dorsal wall of the stomodæum a tongue of cells now extends forward toward the floor of the posterior fore-brain, which has become bent down in front of it. This is the *hypophysis* (Fig. 162).

The Visceral Pouches and Arches

The Pouches. — In the anterior or pharyngeal portion of the fore-gut, a series of vertical folds of the endodermal wall begin to push out toward the ectoderm on each side of the head. These are the *visceral pouches*, and they develop in regular order, the most anterior pair appearing first. The first pair are known as the *first visceral* or *hyomandibular* pouches, and the remaining pairs, of which there are three, as the *second, third*, and *fourth visceral* (" *branchial* ") pouches. They decrease in size posteriorly, the last pair being relatively small. The first pair of pouches; i.e., the hyomandibulars, fuse with the corresponding ectodermal invaginations (*visceral furrows*) only at their dorsal ends, while the second and third pairs fuse with their respective furrows throughout their lengths, except for a slight interruption in their upper halves. The point of fusion of the first pouch now becomes perforated as the first or *spiracular* cleft. The fusion of the fourth pair of pouches and furrows, and the perforation at the points of fusion of the second and third pairs to form actual *visceral clefts*, occurs later (Figs. 154 and 172).

The Arches. — Anterior and posterior to each pouch the mesenchyme becomes thickened to form the *visceral arches*. The arch in front of the first or hyomandibular pouch is the *first visceral* or *mandibular* arch, and the one between it and the second pouch is the *second visceral* or *hyoid arch*. The remainder are simply the *third, fourth,*

and *fifth visceral* (" *branchial* ") arches, and they appear in the same order as the pouches; the fifth and last arch is hardly more than a transitory vestige. Presently, blood vessels and nerves pass into the arches, as will be indicated later.
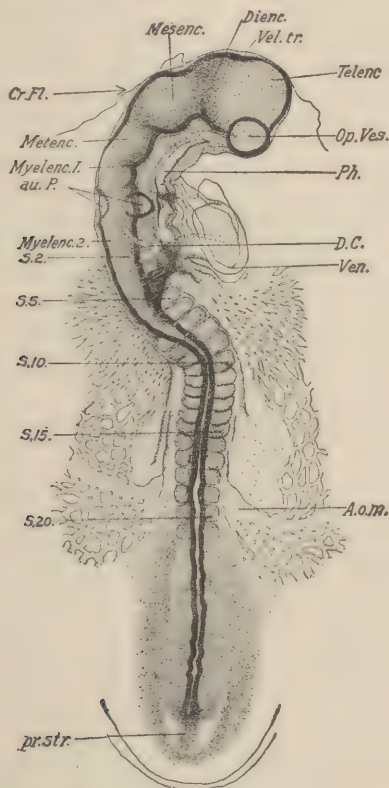
Fig. 153. — Chick embryo with twenty pairs of somites (about forty-three hours). Dorsal view. From Lillie (Development of the Chick).

A.o.m. Vitelline artery. *au.P.* Auditory pit. *Cr.Fl.* Cranial flexure. *D.C.* Ductus Cuvieri. *Dienc.* Diencephalon. *Mesenc.* Mesencephalon. *Metenc.* Metencephalon. *Myelenc. 1* and *2.* Anterior and posterior divisions of the myelencephalon. *Op.Ves.* Optic Vesicle. *Ph.* Pharynx. *pr.str.* Primitive streak. *s.2. s.5.*, etc. Second, fifth, etc., somites. *Telenc.* Telencephalon. *Vel.tr.* Velum transversum. *Ven.* Ventricle.

It should be noted in passing, that though these pouches and arches correspond to the similarly developed structures in the Frog, in this case no gills ever appear in connection with any of them. The term visceral rather than branchial is therefore more aptly applied to them all.

The Thyroid. — This begins to develop near the end of the second day as a small thickening in the middle of the floor of the pharynx, between the ventral ends of the second pair of visceral arches. Before
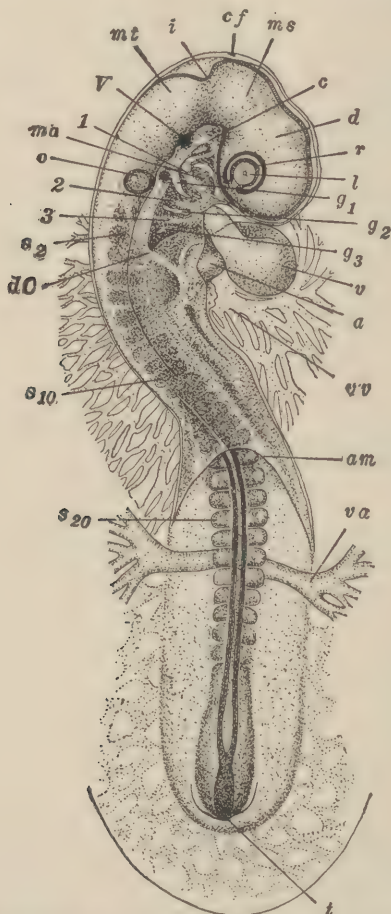
Fig. 154. — Chick embryo with twenty-seven pairs of somites (about forty-eight hours). Viewed from above following lateral rotation of anterior end (see p. 325). From Kellicott (Chordate Development). After Lillie.

a. Auricle. *am.* Posterior margin of amnionic folds. *c.* Carotid loop. *cf.* Cranial flexure (cervical flexure also shown, see p. 324). *d.* Diencephalon. *dC.* Ductus Cuvieri. g_1, g_2, g_3. First, second and third visceral clefts. *i.* Isthmus. *l.* Lens. *ma.* Mandibular arch. *ms.* Mesencephalon. *mt.* Metencephalon. *o.* Otocyst (auditory sac); just to the right of the otocyst is a thickening representing the ganglion of the VII and VIII cranial nerves. *r.* Retinal layer. s_2, s_{10}, s_{20}. Second, tenth, and twentieth somites. *t.* Tail-bud. *v.* Ventricle. *va.* Vitelline artery. *vv.* Vitelline vein. *1, 2, 3.* First, second and third aortic arches. *V.* Ganglion of V cranial nerve.

the end of the day it has become slightly evaginated so as to form a shallow depression in the pharyngeal floor (Fig. 172).

The Respiratory System. — Late the second day a longitudinal groove appears in the floor of the pharynx posterior to the visceral pouches. It is the beginning of an outpushing which will form the larynx, the trachea, and the lungs. It thus represents the beginning of the entire respiratory system.

The Liver. — Just at the posterior limit of the fore-gut behind the pharyngeal region, there appear at this time two slight antero-ventrally directed evaginations of the endoderm. One of these is a little in advance of the other both in position and in time of appearance. It presently pushes forward so as to lie just dorsal to the point of union of the vitelline veins (see below), while the other, at this period, is barely distinguishable. These two diverticula represent the rudiments of the *liver*.

The Mid-Gut

There is little indication of any real mid-gut during the second day, but rather merely a wide enteric space overlying the yolk. The beginning of folds along the sides of the embryo continuous with the lateral margins of the head fold suggest, however, the manner in which this portion of the gut will be formed.

The Hind-Gut

The Posterior Intestinal Portal and Anal Plate. — At the close of the second day the hind-gut begins to develop in connection with a tail fold very similar to the head fold. There is thus formed a posteriorly directed cavity lined by endoderm, and lying beneath the remains of the primitive streak. It is the *hind-gut*, and opens anteriorly into the wide enteric space overlying the yolk (rudiment of the mid-gut). As in the case of the fore-gut, the region of this opening is termed an intestinal portal — in this instance, the *posterior intestinal portal*. There is finally one further resemblance between fore and hind-guts in that at the end of the latter the endoderm comes in contact with the ectoderm and fuses with it. This point of fusion is at the posterior end of the primitive streak, and marks the location of the future anus. It is termed the *anal plate*. Besides these points of re-

semblance, there are now to be noticed certain important differences as follows (Fig. 155):

The Ventral Mesentery. — It has been stated that the hind-gut is formed in connection with a tail fold, just as the fore-gut is formed in connection with the head fold, and in a general way this is true. In the case of the tail fold, however, there is this difference. The endoderm is folded in to form the hind-gut and the intestinal portal, but in this case the ectoderm follows this infolding much more slowly than it did in the case of the head fold. Thus it happens that the hind-gut arises before there is any very marked indication of a tail fold on the surface of the blastoderm. For this reason the anal
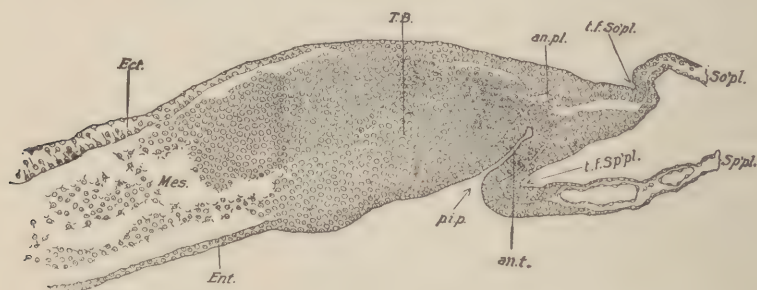
Fig. **155.** — Median longitudinal section through the hind end of an embryo of about **21 s.** From Lillie (Development of the Chick).

an.pl. Anal plate. *an.t.* Anal tube (hind-gut). *Ect.* Ectoderm. *Ent.* Endoderm. *Mes.* Mesoderm. *p.i.p.* Posterior intestinal portal. *T.B.* Tail-bud. *t.f.So'pl.* Tail fold in the somatopleure and ectoderm. *t.f.Sp'pl.* Tail fold in the splanchnopleure and endoderm.

plate, unlike the oral plate, remains dorsal for some time, and is only gradually carried around onto the ventral surface (Fig. 155).

Furthermore, this lagging behind of the ectodermal portion of the fold necessarily means that there is a gap between the two cell layers; this gap in the case of the tail fold is filled by mesoderm. Presently lateral extensions of the embryonic cœlom press back into this region upon either side, but for a time they do not meet one another. Thus there is left a median mesodermal mass extending from the ventral side of the hind-gut backward and upward to the underside of the lagging ectoderm. That portion in contact with the gut may be referred to as splanchnic, and that in contact with the ectoderm as somatic. The two portions are continuous, however, and together are known as the *ventral mesentery* of the hind-gut.

THE CIRCULATORY SYSTEM

THE HEART

The Origin and the Formation of the Endothelial Lining. — While blood vessels and corpuscles have been developing from the germ wall in the area opaca, vessels have also begun to form in the area pellucida. These latter vessels, which are in direct continuity
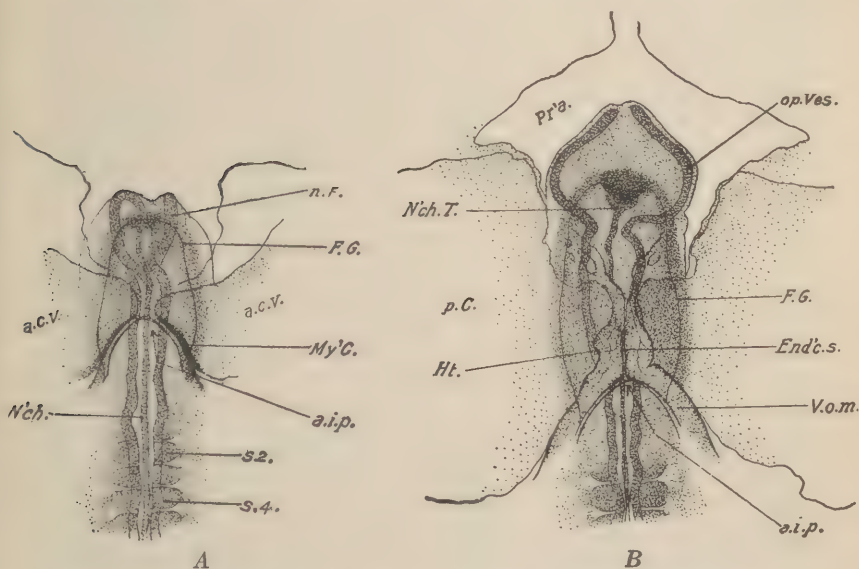
Fig. 156. — Ventral views of the head ends of Chick embryos. From Lillie (Development of the Chick). A. Embryo with five pairs of somites (about twenty-three hours). B. Embryo with seven pairs of somites (about twenty-five hours).

a.c.v. Amnio-cardiac vesicle. *a.i.p.* Anterior intestinal portal. *End'c.s.* Endocardial septum. *F.G.* Fore-gut. *Ht.* Heart. *My'C.* Myocardium. *N'ch.* Notochord. *N'ch.T.* Anterior tip of notochord. *n.F.* Neural fold. *op.Ves.* Optic vesicle. *p.C.* Pericardial cavity (amnio-cardiac vesicles). *Pr'a.* Proamnion. $s_2.s_4.$ Second and fourth mesodermal somites. *V.o.m.* Omphalo mesenteric vein.

with those already formed, also arise from blood islands, though these islands are slightly different from those of the area opaca. They are merely aggregations of cells, apparently detached from the splanchnic mesoderm, and the vessels into which they develop are temporarily entirely devoid of corpuscles. These, however, are soon supplied
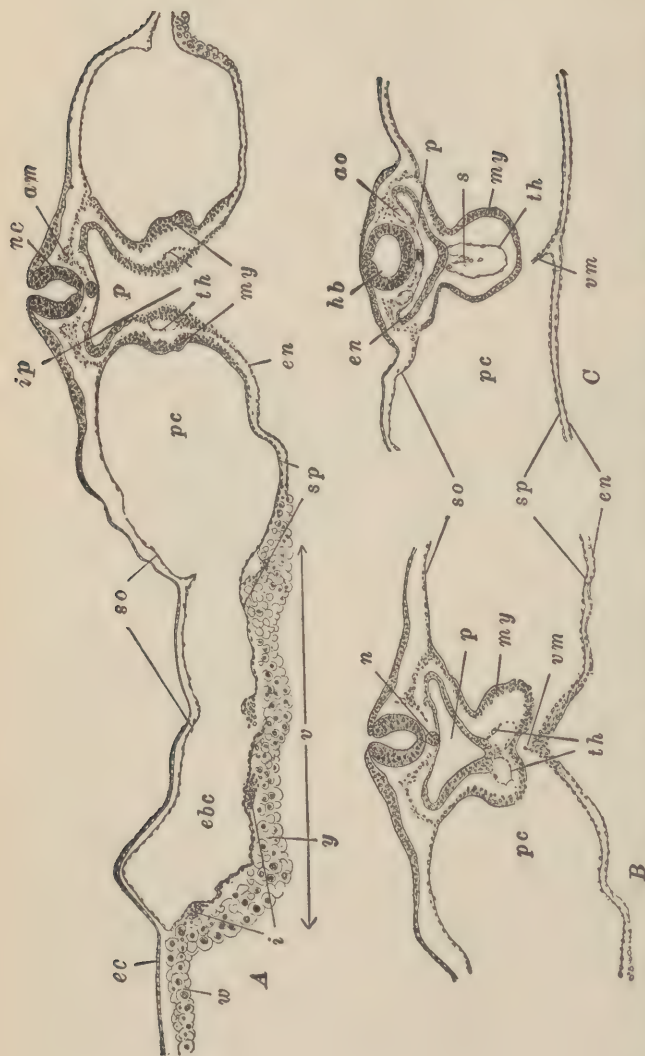
Fig. 157. — Sections through Chick embryos showing particularly the formation of the heart, pericardial cavity, and pharynx. From Kellicott (Chordate Development). After Lillie. A. Just posterior to the anterior intestinal portal of a Chick with seven pairs of somites (about twenty-five hours). B. Section just anterior to A. C. Through the heart of an embryo with ten pairs of somites (about twenty-nine hours).

am. Axial mesodermal thickening. ao. Lateral dorsal aorta. ebc. Exocoelom. ec. Ectoderm. en. Endoderm. hb. Hind-brain. i. Blood islands. ip. Anterior intestinal portal. my. Myocardium (mesodermal or muscular layer of heart). n. Notochord. nc. Nerve cord. p. Pharynx. pc. Pericardial cavity (amnio-cardiac vesicles). s. Endothelial septum. so. Somatic mesoderm. sp. Splanchnic mesoderm. th. Cardiac endothelium. v. Area vasculosa. vm. Ventral mesocardium. w. Germ wall. y. yolk-sac endoderm.

through the connections with the area opaca. In this manner, the rudiments of two large vessels (the *omphalomesenteric* or *vitelline veins*) very soon appear in a particular part of the area pellucida, as follows (Fig. 156): Each rudiment rests upon one of the ventro-lateral walls of the fore-gut, between it and the median-lateral wall of the respective amnio-cardiac vesicle from which it has probably arisen (Fig. 157, *A*).[1] The anterior portions of these rudiments then form the endothelial lining of the heart, in the following manner:

It is to be recalled that the amnio-cardiac vesicles have already become fused beneath the fore-gut, just in front of the endodermal wall which marks its posterior limit (Fig. 156, *A*). The fusion now progresses posteriorly, as it does so pushing back and closing in the ventro-lateral gut walls against which the veins indicated in the preceding paragraph are resting. Thus as these walls come together the anterior ends of the above mentioned vessels are likewise brought together side by side beneath the newly formed gut, and as this occurs they fuse with one another to form a single vessel with a median partition. This partition soon disappears, and the single median tube which remains is the *endothelial lining* of the *rudimentary heart* (Figs. 156, *B* and 157, *B, C*).

The Myocardium of the Heart. — The median walls of the amnio-cardiac vesicles which now lie against each side of the endothelial tube presently press in above and below it, and fuse with each other. Thus the tube is completely surrounded by mesoderm which forms the *myocardium* or muscular element of the heart.

The Mesocardia. — The above fusion leaves the endothelial tube and its myocardium suspended from the mesodermal covering of the ventral wall of the fore-gut, or pharynx, by a double layered sheet of mesoderm, the *dorsal mesocardium*. Ventrally also a similar sheet attaches the tube to the underlying splanchnic mesoderm. The latter quickly disappears, and the former does so later, except at the anterior and posterior ends of the heart (Fig. 157, *C*).

The Pericardial Cavity and Pericardium. — With the fusion and disappearance of the median walls of the amnio-cardiac vesicles, it is clear that their cavities have become a single space which surrounds

[1] The evidence of this figure would seem to indicate that the vessels are derived from the walls of the gut rather than from those of the vesicles, and some authorities hold this to be the case. In view, however, of the origin of the other blood vessels of this area from the mesoderm, it seem more likely that the latter derivation is the true one.

the heart. This space is the *pericardial cavity,* and its walls constitute the rudiments of the greater part of the *pericardium.* Postero-laterally, however, the pericardium is still incomplete, and hence the above cavity continues to communicate in this direction with the general cœlom.

The Rudiments of the Auricles, Ventricles, and Bulbus Arteriosus. — Within the pericardial cavity between the anterior and the posterior points of mesocardial attachment, the tubular heart rapidly increases in length. This increase in length presently results in an outward bending to the right of the median line (Fig. 158). The posterior limit of this outward bend will be designated as *A,* the
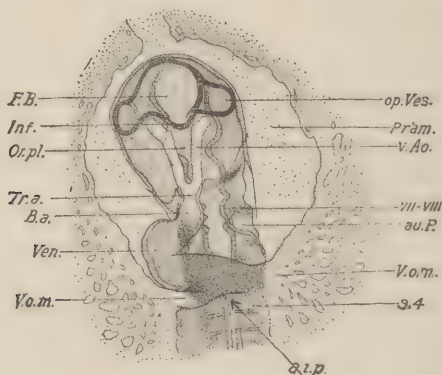
Fig. **158.** — Ventral view of the anterior end of a Chick embryo with sixteen pairs of somites (about thirty-eight hours). From Lillie (Development of the Chick).
a.i.p. Anterior intestinal portal. *au.P.* Auditory pit. *B.a.* Bulbus arteriosus. *F.B.* Fore-brain. *Inf.* Infundibulum. *op.Ves.* Optic vesicle. *Or.pl.* Oral plate. Pr'am. Proamnion. *s.4.* Fourth somite. *Tr.a.* Truncus arteriosus. *v.Ao.* Ventral aorta. *Ven.* Ventricle. *V.o.m.* Omphalomesenteric (vitelline) vein. *VII–VIII.* Acustico-facialis ganglion.

apex of the bend or loop as *B,* and the point at which the anterior limb of the loop rejoins the middle line as *C.*

As growth continues, *B,* the rather broad apex of the loop, moves backward and somewhat ventrally; this backward and ventral movement persists until the apex has been carried to the median line beneath and a little posterior to the point *A.* Thus the posterior limb of the originally horizontal loop now extends ventrally and slightly backward from *A* to *B.* Let there next be imagined upon the anterior limb of the loop (i.e., the limb *BC*) a new point *D,* somewhat nearer to *B* than to *C.* By a twist in this limb which accompanies the above

mentioned bendings, the posterior portion of it; i.e., the portion *BD*, now extends outward to the right from *B* in an approximately transverse position. From *D*, upon the other hand, the more anterior part of this anterior limb; i.e., the part *DC*, bends upward and inward to the point *C* in the median line.

At the point *A* (*atrium*, Fig. 162) two lateral pockets appear, the left being the larger; they are the rudiments of the *auricles* (Fig. 154). The loop now running downward from *A* to *B* and outward to the right from *B* to *D*, is entirely *ventricular,* as is also the first portion of the section running upward and inward from *D* to *C*. The remainder of the last named section, however, is separated from the first or ventral portion by a slight constriction which marks the end of the ventricular region. Following this constriction, the ascending tube is somewhat dilated to form the *bulbus arteriosus* (Fig. 158), whose anterior connection will be described below. Rhythmical contractions begin in the heart toward the end of the second day.

THE BLOOD VESSELS OF THE EMBRYO

The Arteries.

The Dorsal Aortæ and Their Branches. Along each side of the embryo, just at the inner margin of the pellucid area, two vessels now develop. These are the *dorsal aortæ* (Fig. 159). Anteriorly each is continued into a vessel differentiated in the mesenchyme on either side of the head. Posteriorly they give off branches between the somites (*segmental arteries*), and finally leave the sides of the embryo at about the level of the seventeenth somite to pass out into the general vascular network as the *vitelline arteries*. Near the end of the second day the two dorsal aortæ fuse with one another in the region above the heart, forming for a short distance a single dorsal vessel.

The Ventral Aorta and the Aortic Arches. The bulbus arteriosus is continuous anteriorly with a large short vessel which passes forward and upward beneath the fore-gut. It is the *ventral aorta* or *truncus arteriosus* (Fig. 158). Near its anterior termination this vessel divides into two parts, which at about the level of the oral plate pass upward around the sides of the head within the substance of the mandibular arches. Dorsally they join the dorsal aortæ. By the end of the second day similar vessels with similar connections have formed in the second visceral or hyoid arches and also in the third visceral arches.
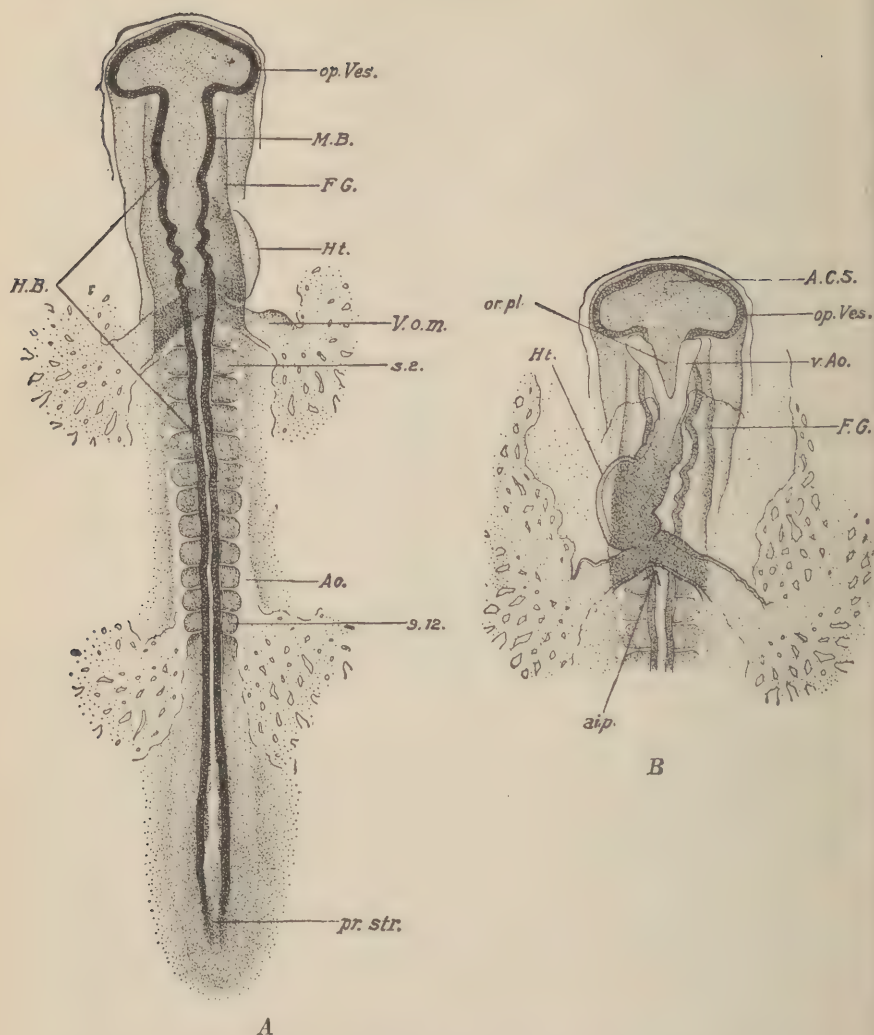
Fig. 159. — Chick embryo with twelve pairs of somites (about thirty-three hours). From Lillie (Development of the Chick). A. Dorsal view of entire embryo. B. Ventral view of anterior end.

A.C.S. Anterior cerebral suture. *a.i.p.* Anterior intestinal portal. *Ao.* Dorsal aorta. *F.G.* Fore-gut. *H.B.* Hind-brain. *Ht.* Heart. *M.B.* Mid-brain. *op.Ves.* Optic vesicle. *or.pl.* Oral plate. *pr.str.* Primitive streak. $s_2.s_{12}.$ Second and twelfth somites. *v.Ao.* Ventral aorta. *V.o.m.* Omphalomesenteric vein.

These vessels are known respectively as the *first, second,* and *third* pairs of *aortic arches* (Fig. 154).

The Veins and the Lateral Mesocardia. — As has been indicated above, the endothelial portion of the heart is formed by the growing together of two large vessels (omphalomesenteric veins). It now remains to state that this union continues for a short distance posterior to the auricular rudiments (atrium). The most anterior part of this continuation is somewhat dilated and is known as the *sinus venosus,* while slightly further back it receives the name of *ductus venosus.* The most anterior portion of the sinus venosus is sometimes regarded as part of the heart proper, because later it is involved in the development of the right auricle. At this stage, however, it may best be considered as a part of the venous system.

During the second day there develops in the mesenchyme on each ventro-lateral side of the brain a vessel which runs posteriorly as far as the level of the heart. These are the *anterior cardinal veins.* At this point each anterior cardinal joins the dorsal end of a vein running upward and outward from the sinus venosus. From the point of union with the new vein the former vessel then continues backward along the dorso-lateral side of the embryo nearly to the tail, as a *posterior cardinal.* The vein which unites the cardinal with the sinus venosus is a *ductus Cuvieri* (Fig. 154), and it should be noted in this connection that each ductus Cuvieri passes from its origin to its junction with the cardinal vein along a newly developed septum. These septa lie just back of the heart and, as their relations to the ductus indicate, extend from the base of each vitelline vein (see below) obliquely upward to the dorso-lateral body wall. They are the *lateral mesocardia,* and, as will appear below, help to separate the pericardial cavity from the general cœlom (Fig. 179).

The Extra-Embryonic Blood Vessels

Extension of the Area Vasculosa and the Mesoderm. — By about the end of the second day the two anterior wings of the area vasculosa, and the extra-embryonic mesoderm and entoderm which accompany them, have bent toward one another and fused in front of the proamnion. The area vasculosa, therefore, now entirely surrounds the latter region, and is itself completely encircled by the sinus terminalis referred to above (Fig. 160, *A, B*). Meanwhile, certain veins and arteries have extended from the embryo into the vascular area, as follows:
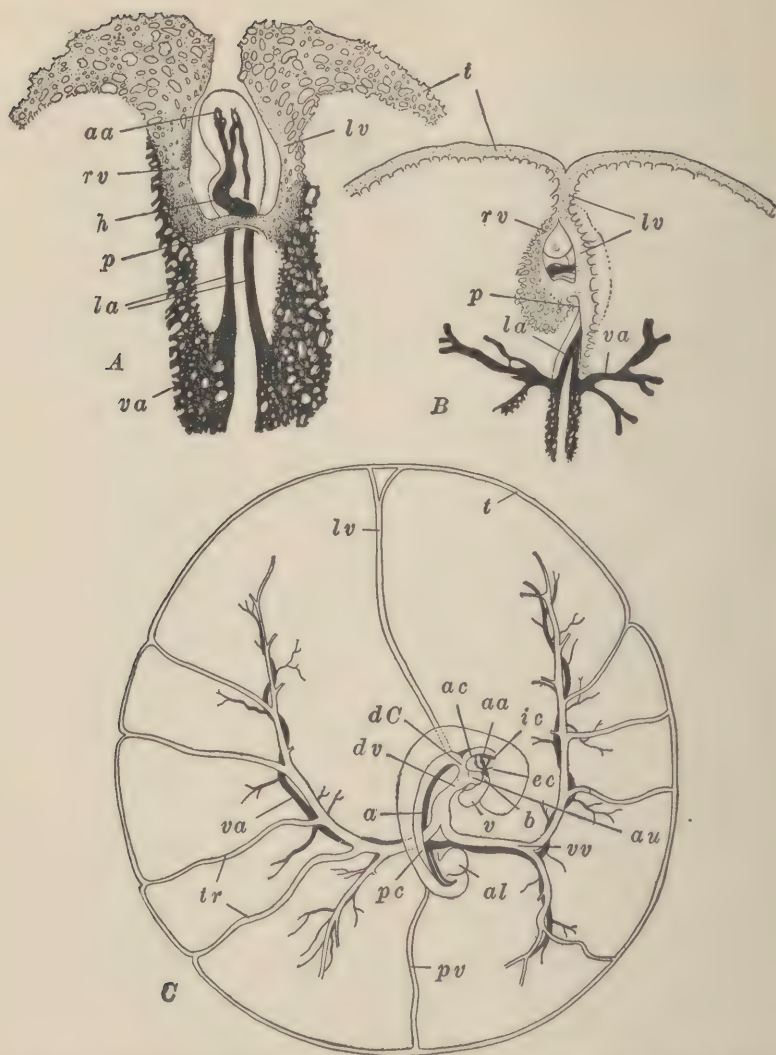
Fig. 160. — Diagrams of the circulation in the Chick embryo and area vasculosa. From Kellicott (Chordate Development). The vascular network of the area vasculosa is omitted for the most part. A. Anterior and central parts of the embryo and vascular area at about thirty-eight hours (sixteen pairs of somites). Viewed from beneath. After Popoff. B. Median and anterior parts of vascular area and embryo at about seventy-two hours (twenty-seven pairs of somites; the number is usually nearer to 36 at this age). Viewed from beneath. After Popoff. C. The main vascular trunks of the fourth day. After Lillie (modified).

a. Dorsal aorta. *aa*. Aortic arches (first and second in A, second, third and fourth in C). *ac*. Anterior cardinal vein. *al*. Allantois. *au*. Auricle. *b*. Bulbus arteriosus. *dC*. Ductus Cuvieri. *dv*. Ductus venosus. *ec*. External carotid artery. *h*. Heart. *ic*. Internal carotid artery. *la*. Lateral dorsal aorta. *lv*. Left anterior vitelline vein. *p*. Anterior intestinal portal. *pc*. Posterior cardinal vein. *pv*. Posterior vitelline vein. *rv*. Right anterior vitelline vein. *t*. Sinus terminalis. *tr*. Venous trunks of the area vasculosa. *v*. Ventricle. *va*. Vitelline artery. *vv*. Vitelline or omphalomesenteric vein (in this region really lateral vitelline vein).

At the posterior end of the ductus venosus, the union of the vessels which form it terminates, and each passes outward into the area pellucida. At this point they are known as the vitelline or omphalo-mesenteric veins. Upon coming into this region each of the veins turns anteriorly and runs past the head around the inner boundaries of the approaching wings of the area vasculosa. Hence these extensions are known as the right and left *anterior vitelline veins*. First by a system of capillaries, but presently directly, each of these veins then becomes connected with the anterior extremities of the sinus terminalis. It thus happens that as the vascular wings meet one another, the sinus terminalis not only becomes complete, but the ends of the two anterior vitelline veins also meet and form one vessel (Fig. 160, *B*). At the proximal ends of these veins each gives rise during this period to a slight lateral outgrowth — the beginnings of the *lateral vitelline veins*.

The vitelline arteries, already referred to, extend out into the lateral portions of the area vasculosa some distance back of the vitelline veins; i.e., at about the twentieth somite.

THE CIRCULATION AS ESTABLISHED ON THE SECOND DAY

It will now be seen that with the establishment of the capillary network within the area vasculosa, and the formation of the arches connecting the ventral and dorsal aortæ within the embryo, a complete system of circulation has been made possible. The further development of this system will be described as it occurs.

THE NERVOUS SYSTEM

THE MAIN DIVISIONS OF THE EARLY BRAIN

Early on the second day of incubation a slight constriction appears just back of the optic vesicles, marking the posterior boundary of the *fore-brain* or *prosencephalon*. Presently this is followed somewhat further back by another constriction which marks the posterior limit of the *mid-brain* or *mesencephalon*. The part posterior to this is the *hind-brain* or *rhombencephalon* which passes insensibly into the region of the spinal cord. The posterior limit of the hind-brain, however, may be fixed in a general way at this time by the position of the fourth somite (Fig. 161).

The Flexures of the Brain and Embryo

The various flexures which are to be described under this heading affect not only the nervous system, but the entire embryo. This is a point which should be kept clearly in mind. However, inasmuch as the nervous system takes so prominent a part in giving shape to the embryo at this stage, it is convenient to follow the progress of the changes in terms of this system.
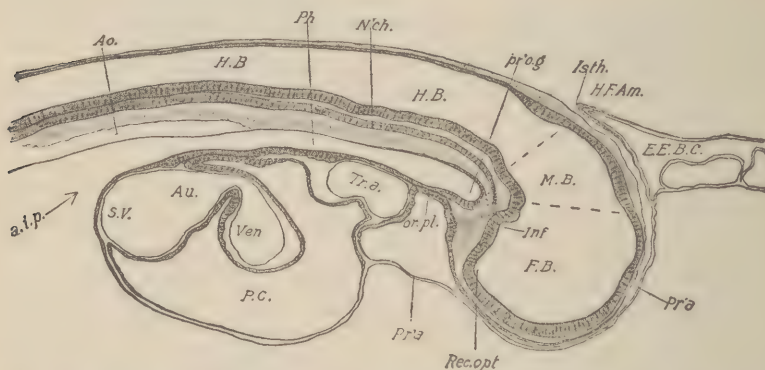
Fig. 161. — Median sagittal section through the head end of a Chick with eighteen pairs of somites (about forty hours). From Lillie (Development of the Chick).

a.i.p. Anterior intestinal portal. *Ao.* Dorsal aorta. *Au.* Auricle. *E.E.B.C.* Exocœlom (extra-embryonic body cavity). *F.B.* Fore-brain. *H.B.* Hind-brain. *H.F.Am.* Head-fold of amnion. *Inf.* Infundibulum. *Isth.* Isthmus. *M.B.* Mid-brain. *N'ch.* Notochord. *or.pl.* Oral plate (oral membrane). *P.C.* Pericardial cavity. *Ph.* Pharynx. *Pr'a.* Proamnion. *pr'o.g.* Preoral gut. *Rec.opt.* Optic recess. *S.V.* Sinus venosus. *Tr.A.* Truncus arteriosus. *Ven.* Ventricle.

The Cranial Flexure. — At about the thirtieth hour, a downward flexure of the embryo begins in the vicinity of the mid-brain, which results in bringing this region into a position approximately anterior to the end of the notochord. This movement at the same time brings the fore-brain to a position partially beneath the notochord, with its originally anterior end facing postero-ventrally. The bend which is thus produced is known as the *cranial flexure* (Figs. 154 and 162).

The Cervical Flexure. — At about the end of the second day, the second general downward flexion begins, involving the entire region of the hind-brain and the neck. It is not so sharp as the first, but more extensive, and is known as the *cervical flexure* (Fig. 154).

The Lateral Rotation. — These flexures would result in thrusting the anterior portion of the embryo deep down into the yolk, were it not for a third process which goes on at the same time. This is a lateral rotation or twisting of the anterior part of the body of the embryo, such that the entire brain region soon comes to lie upon its left side (Fig. 154). Having thus indicated the new position of the brain, we are now prepared to describe its further development during the second day. The terms dorsal, ventral, and lateral in the present and following descriptions of this and other systems are used in their morphological sense; i.e., they are used as though the lateral twist had not occurred. This also applies to the foregoing account of the heart.

The Fore-Brain or Prosencephalon

Its Extent. — On the posterior wall; i.e., the floor of the flexed portion of the brain, at the end of the slightly bent notochord, is an evagination directed antero-ventrally into the cavity of the brain. This is the *tuberculum posterius* (Fig. 162). On the opposite or anterior wall of the brain a little below the level of this evagination is a thickening which marks the position of the future posterior commissure (see fourth day). A line drawn from the tuberculum posterius to the thickening on the anterior wall will lie in the plane which marks the posterior limit of the prosencephalon or fore-brain. As already noted, this limit is marked externally by a slight constriction.

Its Parts.
The Infundibulum. — Just ventral to the tuberculum posterius, a small posteriorly directed evagination now appears lying slightly beneath the anterior end of the notochord. It is the beginning of the *infundibulum* (Fig. 162).

The Region of the Optic Vesicles. — Ventral to the infundibulum, but still on the posterior wall, is a thickening, the rudiment of the future *optic chiasma* (not noticeable in Fig. 162), while immediately ventral to this thickening is a small evagination, the *optic recess*. From this recess the hollow optic vesicles have grown out on either side, and as they have grown their proximal parts have been constricted, as in the case of the Frog, to form the *optic stalks*. Below the optic recess, the posterior wall begins to curve anteriorly on to the present ventral surface. This region is relatively thin and is known

as the *lamina terminalis.* Within it the torus trasversus is scarcely visible as yet.

The Cerebral Hemispheres. Near the end of the second day the sides of the fore-brain just dorsal to the lamina terminalis begin to push out as the future *cerebral hemispheres.* Their cavities will be the *lateral ventricles* opening into the cavity of the fore-brain or *third ventricle,* through the *foramina of Monro.*
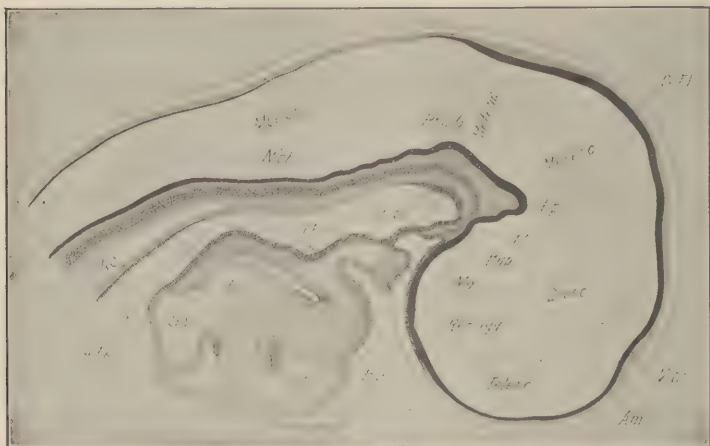
Fig. 162. — Optical sagittal section of the head of an embryo of 22–23 s. The heart is represented entire. From Lillie (Development of the Chick).

Atr. Atrium. *Ba.* Bulbus arteriosus. *Cr.Fl.* Cranial flexure. *Dienc.* Diencephalon. *Hyp.* Hypophysis. *Inf.* Infundibulum. *Md.* Mandibular arch. *Metenc.* Metencephalon. *Myelenc.* Myelencephalon. *or.pl.* Oral plate. *Pr'o.G.* Preoral gut. *Th.* First indication of thyroid. *Rec. opt.* Optic recess. *Telenc.* Telencephalon. *T.p.* Tuberculum posterius. *V.tr.* Velum transversum.

The Velum Transversum and Region of Epiphysis. Beyond the region of the lamina terminalis on the antero-ventral side of the fore-brain, we come to a portion of the wall which is slightly depressed. It is known as the *velum transversum.* Further dorsal to this point on approximately the anterior surface may be found, also, the suggestion of an outpushing; it marks the general region from which the epiphysis (pineal gland) later (fourth day) arises. This brings us to the thickening mentioned above as indicating the location of the posterior commissure, and the limit of the fore-brain.

The Divisions of the Fore-brain. — As in the case of the Frog, it is customary to divide the fore-brain into two parts, which with the

aid of the above landmarks may now be easily defined. That part of
the fore-brain which lies ventral to a plane passing from the posterior
wall just ventral to the optic recess to the anterior wall about the
middle of the velum transversum is the *telencephalon*. The remaining
portion of the fore-brain, whose posterior limit is defined above, is then
the *diencephalon*. The cerebral hemispheres arise from the former.

THE MID-BRAIN OR MESENCEPHALON

The anterior boundary of the mesencephalon coincides with the
posterior boundary of the diencephalon, marked by the external con-
striction previously referred to. The posterior boundary may be de-
fined as a transverse plane passing from the postero-ventral wall or
floor just above and behind the tuberculum posterius, upward to about
the middle of a rather broad constriction on the antero-dorsal wall
(Fig. 162). The roof of the mid-brain, moreover, is growing so
rapidly in connection with the cranial flexure, that it soon arches out-
ward as the most anterior region of the embryo. Other parts of
the mesencephalon have not appeared, and will, therefore, be de-
scribed later as they arise.

THE HIND-BRAIN OR RHOMBENCEPHALON

Its Extent. — The hind-brain lies entirely dorsal to the notochord,
and extends from the constriction marking the boundary of the mid-
brain posteriorly into the spinal cord. Its posterior boundary, as
stated above, can be defined only as that part opposite the fourth
somite. As in the case of the mid-brain, the parts of the hind-brain
are not yet discernible, and will be indicated when they appear.

The Divisions of the Hind-brain. — The divisions of the hind-
brain are also difficult to define at this early stage. We may say,
however, that the anterior division is relatively short, and is known as
the *metencephalon*. The remainder of the brain constitutes the pos-
terior division known as the *myelencephalon*. The cavity which extends
through both is called the *fourth ventricle*.

THE SPINAL CORD AND ITS NEURAL CRESTS

The Cord. — As fast as the neural tube is formed by the fusion
of the neural folds, its central canal tends to become compressed
laterally and elongated dorso-ventrally. Its lateral walls also gradually

thicken, and at the end of the second day these walls consist chiefly of two sorts of cells. First, there are elongated cells extending from the central canal out to its outer walls. These are the original ectodermal elements, now known as *ependymal cells,* and their function is that of support. Secondly, among the ependymal cells and near the central canal are numerous rounded cells known as *germinal cells.* They later give rise to *neuroblasts* or primitive nerve cells, and also probably to more supporting elements termed *glia cells.*

The Neural Crests and Rudimentary Spinal Ganglia. — As indicated in the previous chapter, the neural crests when first formed are simply bands of cells which extend along the dorso-lateral walls of the neural tube, on either side between it and the ectoderm. As was also stated, these bands or crests are at first fused with one another dorsally. By the end of the second day, however, in the older (i.e., anterior) portion of the tube, this dorsal fusion has been obliterated. In this region there have also appeared in the crests successive enlargements, which presently become separated from one another to form a series of *rudimentary spinal ganglia.* There is one of these ganglia for each somite, except for those of the head region, opposite whose somites the crests disappear. The spinal ganglia at this time contain both neuroblasts and indifferent cells.

The Cranial Ganglia

The neural crests of the head region anterior to the somites do not disappear, but also form enlargements which separate to constitute the cranial ganglia. By the end of the second day these are visible, beginning at the anterior end, in the following positions:

The V Nerve Ganglion. — The ganglion for the V or *trigeminal* nerve is somewhat anterior to the dorsal end of the first or mandibular arch. At the end of the second day it usually appears merely as a dark patch in this region (Fig. 154), but later (see third day) it acquires distinctly the form of an inverted Y.

The VII and VIII Nerve Ganglia. — The ganglia for these nerves form a single mass, the *acustico-facialis* ganglion. It lies at this time just antero-ventral to the auditory sac (see below); i.e., it is above and slightly in front of the dorsal end of the second or hyoid arch. Though unlabeled, it is shown in Fig 154 in the position indicated.

The IX and X Nerve Ganglia. — The IX and X nerve ganglia

arise together, but at the end of the second day they begin to become separated. The former, or *glosso pharyngeal* ganglion, is then situated above the dorsal end of the third visceral arch while the latter, or

vagus ganglion, lies above the ends of the fourth and fifth visceral arches. These ganglia are not visible in Fig. 154. A diagram of the general location and form of all the cranial ganglia viewed from above early on the second day is given in Fig. 163.

ORGANS OF SPECIAL SENSE

THE EYE

The Optic Stalks, the Optic Cup and the Choroid Fissure. — The *optic vesicles,* it will be recalled, are hollow out-pushings from the forebrain with which they remain connected by constricted regions known as the *optic stalks* (Fig. 164). These stalks are the so-called " optic nerves," though as will appear, the real optic nerves develop later. It is to be noted that the above con-

Fig. 163. — Diagram of the cephalic neural crest of a chick of about 12 somites. From Lillie (Development of the Chick). After Wilhelm His. *ot.* Auditory sac. *s.* Somite.

striction has occurred in such a manner that each stalk connects with its vesicle near the ventral side of the latter, rather than at its center. Invagination of the outer wall of the vesicle now occurs, obliterating its original cavity, and converting it into the two-layered *optic cup,* with the optic stalk attached to its ventral edge. The walls of the cup on either side of the point where the stalk is attached now grow outward; i.e., toward the ectoderm, but their ventral edges do not quite meet one another. Thus a fissure is left in the ventral side of the cup extending from its edge inward to the optic stalk. This, as in the Frog, is the *choroid fissure.* Meanwhile the rim of the cup bounding its aperture, the *pupil,* becomes slightly constricted. The invaginated or outer wall of the vesicle has now necessarily become the wall

which lines the cavity of the cup; i.e., its inner wall, and will, therefore, be referred to as the inner wall in future discussion. It is largely the rudiment of the future *retina* (see Chapter XI).

The Development of the Lens. — Before the above invagination of each optic vesicle occurred, the vesicle had pushed out far enough to touch the surface ectoderm. When this happened, the ectoderm at the point of contact began to thicken, and when the invagination of the vesicle took place, this thickened ectodermal wall also invaginated. In this manner, a hollow thick-walled sac was formed resting just
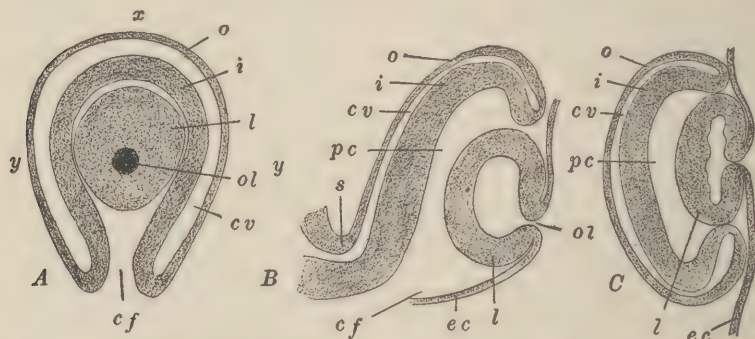
Fig. 164. — Diagrams of sections through the eye of the Chick embryo at the end of the second day. From Kellicott (Chordate Development). After Lillie. The dorsal margin is toward the top of the page in A and B. A. Eye as viewed directly. B. Vertical section through the line x–cf, in A. C. Horizontal section through the line y–y in A.

cf. Choroid fissure. *cv*. Cavity of primary optic vesicle. *ec*. Superficial ectoderm of head. *i*. Inner or retinal layer of optic cup. *l*. Lens. *o*. Outer or pigmented layer of optic cup. *ol*. Opening of lens sac from surface of head. *pc*. Posterior chamber of eye. *s*. Optic stalk, continuous with the floor and lateral wall of the diencephalon.

within the rim of the optic cup. This is, of course, the rudiment of the *lens;* at the end of the second day it has not quite detached itself from the outer ectoderm.

THE EAR

The sensory part of the ear begins as a thickening of the ectoderm on the side of the head above and slightly posterior to the dorsal end of the hyoid arch. This thickening presently starts to invaginate, thus forming a depression — the *auditory pit.* During the second day the process of invagination continues, and is soon accompanied by an approximation of the anterior and posterior lips of the pit. Near the

end of the second day the ventral lip also takes part in the closure by moving dorsally, and thus the pit is transformed into a small mouthed sac. It is the *auditory sac* or *otocyst* (Fig. 154).

THE URINOGENITAL SYSTEM

Because of their close connection in the adult, the excretory and reproductive systems are, as usual, considered under a common heading. Their development, however, is largely separate, and must, therefore, be so treated. Of the two systems, only certain parts of the excretory appear during the second day.

THE EXCRETORY SYSTEM

The excretory system of the Chick in common with that of other Amniota consists of three separate parts, the *pronephros, mesonephros,* and *metanephros*. These parts develop in the order named, and the first two have largely disappeared by the close of embryonic life; only the last remains functional as the permanent excretory organ of the adult. During the second day the pronephros develops, and near its close the mesonephros has just begun to appear.

The Pronephros. — The pronephros is vestigial in character, and only appears typically from the tenth to the fifteenth somites. Rudiments of it, however, are sometimes found as far forward as the fifth somite. In the more posterior region indicated, its development is as follows:

The Pronephric Tubules. — In the dorso-lateral portion of the nephrotome opposite the posterior end of each somite a thickening occurs, and from it a cord of cells grows outward and upward for a short distance (Fig. 165, *prn.* 1). At the same time the nephrotome becomes detached from the somite. These lateral outgrowths are termed the *pronephric tubules,* though they usually do not acquire any lumen. Sometimes, however, a slight lumen is present in the proximal part of the tubule (Fig. 165, *prn.* 2), and it opens into the cœlom as a rudimentary *nephrostome.* It is also said that degenerate *glomeruli* (or perhaps more properly *glomi*) sometimes develop later on the cœlomic wall opposite the nephrostomal mouths (Lillie).

The Pronephric and Wolffian Ducts. — The distal part of each of the above cell cords or " tubules " presently bends posteriorly and grows in this direction until it comes in contact with the tubule following it.

In this manner, a continuous backwardly directed cord of cells is formed which connects with each successive tubule. Finally, the bent portion of the last cell cord continues to grow posteriorly between the nephrotomal mass and the body wall. As will appear subsequently, the anterior end of this backward growing rod of cells is the rudiment of the *pronephric duct*, and its more posterior portion, the rudiment
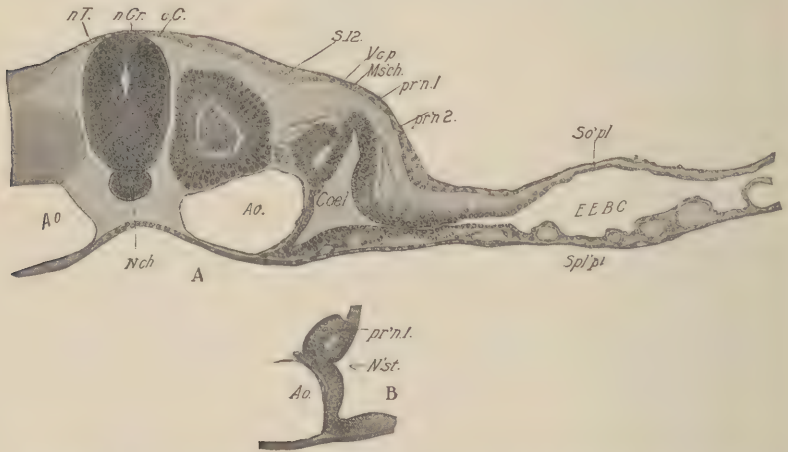
Fig. 165 — A. Transverse section through the twelfth somite of a 16s embryo. From Lillie (Development of the Chick). B. Three sections behind A to show the nephrostome of the same pronephric tubule.

Ao. Aorta. *cC.* Central canal. *Coel.* Cœlom. *E.E.B.C.* Extra-embryonic cœlom (exocœlom). *Ms'ch.* Mesenchyme. *Nch.* Notochord. *n.Cr.* Neural crest. *N'st.* Nephrostome. *n.T.* Neural tube. *pr'n. 1,2.* Distal and proximal divisions of pronephric tubule. *S.12.* Twelfth somite. *So'pl.* Somatopleure. *Spl'pl.* Splanchnopleure. *V.c.p.* Posterior cardinal vein.

of the *mesonephric* or *Wolffian duct*. Before the end of the second day, indeed, the anterior or pronephric section of the rod has acquired a lumen, thus becoming a real duct.

The Mesonephros. — The mesonephros corresponds to the organ of the same name which functions as the permanent excretory organ of the Frog. In the Chick, however, as indicated above, this excretory function continues only during a part of embryonic life. The anterior end of the mesonephros slightly overlaps the posterior end of the pronephric region, but its development here is rudimentary, the organ acquiring its typical form only from the twentieth to the thirtieth somites. During the close of the second day it begins to appear in the following manner, development progressing posteriorly.

The Primary Mesonephric Tubules. — The nephrotome in the region indicated becomes separated both from the somites and the lateral plate. It then lies just ventro-medially to the rod of cells which is to become the Wolffian duct. Above this duct the posterior cardinal vein presently appears, while between the nephrotome and the median line of the embryo runs the dorsal aorta. The nephrotome is thus between the aorta and the future Wolffian duct (Fig. 152). Presently in the neighborhood of each somite, there appear in this nephrotomal band two or more spherical condensations. Then beginning at the anterior end of the band each of these condensed spheres starts to acquire a cavity, each vesicle thus formed being the rudiment of a *mesonephric tubule* and a *Malpighian body*. The more ventral spheres in each somite are the first thus to become vesicular, and they are the rudiments of the so-called *primary mesonephric tubules* as distinguished from the others (Fig. 184. See next chapter).

THE AMNION AND OTHER EXTRA–EMBRYONIC STRUCTURES

From the embryological point of view all Vertebrates belong to one of two classes; i.e., the *Anamniota* or the *Amniota*. The former group includes Amphibians and Fishes, while the latter includes Reptiles, Birds, and Mammals. The Amniota, as the name implies, are those which possess an amnion, while the Anamniota are those which lack it. Amphioxus, the Frog, and Fish have been studied as representatives of the latter class, and we are now studying the Chick as an example of the former or Amniote group. The amnion begins to form on the second day of the Chick's incubation, but is not completed until about the fourth day. In order to make the structure of this organ more clear, however, it seems best to describe its entire development at this time, together with that of certain other extra-embryonic organs and membranes.

THE AMNION IN PROCESS OF DEVELOPMENT

Development During the Second Day. — During the second day a fold in the blastoderm occurs just in front of the head of the embryo in the region of the proamnion. Since there is as yet no mesoderm in this region, the fold at first contains only ectoderm and endoderm. Presently, however, the mesoderm extends into this vicinity, and here,

as elsewhere, is split into the extra-embryonic extensions of the somatic
and splanchnic layers with the extra-embryonic cœlomic space between
them; both these layers then become involved in the fold. The splanch-
nic layer together with the endoderm, however, is soon withdrawn
to the surface of the yolk, while the somatic layer and the extra-embry-
onic ectoderm which covers it constitute the two permanent layers
of the *amniotic head fold*. The embryo has now begun to sink some-
what into the surface of the yolk, and as it does so the amniotic fold
gradually grows back over it. This backward growth is also accom-
panied by the development of *lateral amniotic folds* extending pos-
teriorly on either side. By the end of the second day the embryo has
been covered over in this manner almost as far back as the vitelline

Fig. 166. — Diagrammatic transverse section through the region where the gut is
open out over the yolk (yolk-stalk umbilicus), in a Chick of about forty-eight hours (about
twenty-eight pairs of somites). From Kellicott (Chordate Development). After Duval.
a. Dorsal aorta. *c.* Cœlom. *ebc.* exocœlom. *ig.* Intestinal groove. *la.* Lateral folds
of amnion. *vv.* Vitelline vein.

arteries (Figs. 154 and 166). The latter figure shows a cross section
through a region where the folds have not yet quite covered the embryo.

Development During the Third Day. — About the end of the
second day, or the beginning of the third, another fold appears at the
posterior end of the embryo, and grows forward toward the head fold.
This is the *amniotic tail fold*, which soon becomes coëxtensive upon
either side with the posterior ends of the lateral amniotic folds. It
is similar to the corresponding head fold except that from the first it
contains only ectoderm and somatic mesoderm. Since the anterior
portion of the amnion starts earlier and grows rapidly, the point at
which the converging folds finally meet and fuse is quite near the
posterior end of the animal. The oval opening existing above the
Chick previous to the closure is the *amniotic umbilicus*.

Development During the Fourth Day. — The end of the third, or beginning of the fourth day, marks the meeting and fusion of the amniotic folds at the center of the amniotic umbilicus. The embryo has by this time turned upon its left side throughout the greater part of its length, and inasmuch as the folds do not turn with it, the closure occurs not above its back, but above its right side. It also follows from this, that the fold of the left side covers the back of the embryo as well as a part of the right side. The amnion may now be said to be complete.

The Completed Amnion and Related Parts

The Amnion and Amniotic Cavity. — It is obvious that the amniotic folds, like any other folds, must be composed of two main parts, each part being continuous with the other at the crest of the fold. It is also obvious that one of these parts; i.e., the inner or lower one, lies everywhere next to the embryo. When fusion occurs, therefore, this inner part will become continuous, completely bounding a new cavity which surrounds the embryo at every point except for a restricted region on its ventral side (see below under somatic umbilicus). This continuous inner membrane is the *amnion*, and the cavity thus formed is the *amniotic cavity*. Moreover, inasmuch as the folds involved both ectoderm and mesoderm, the inner membrane or amnion must likewise consist of ectoderm and mesoderm, the former lining the amniotic cavity and the latter forming a coat outside the lining (Figs. 167 and 168).

The Chorion. — At the fusion of the folds the outer part, like the inner, necessarily, becomes continuous. Likewise, it too consists of both ectoderm and mesoderm, but in this case, the ectoderm will lie outside and the mesoderm inside; i.e., toward the amnion. The outer membrane thus constituted is called the *chorion* or *false amnion*. Between it and the inner membrane or true amnion, there is naturally the same space which separated the inner and outer parts of the amniotic folds: i.e., the extra-embryonic cœlom or *exocœlom*. This relationship will be made clear by reference to figure 167. It may be mentioned incidentally in this connection that this exocœlomic space eventually becomes filled by an important sac-like organ (*allantois*) whose origin and structure will be described below.

The Sero-Amniotic Connection. — It has been implied that the

extra-embryonic cœlom, with whatever may occupy it, everywhere separates the amniotic membrane from the chorionic membrane. This is true except at one point. At the point of final fusion of the amniotic folds; i.e., the amniotic umbilicus, the cœlomic space is interrupted by a small area of mesoderm which persists, and serves to unite the above membranes. It is called the *sero-amniotic connection* (Figs. 167 and 168).
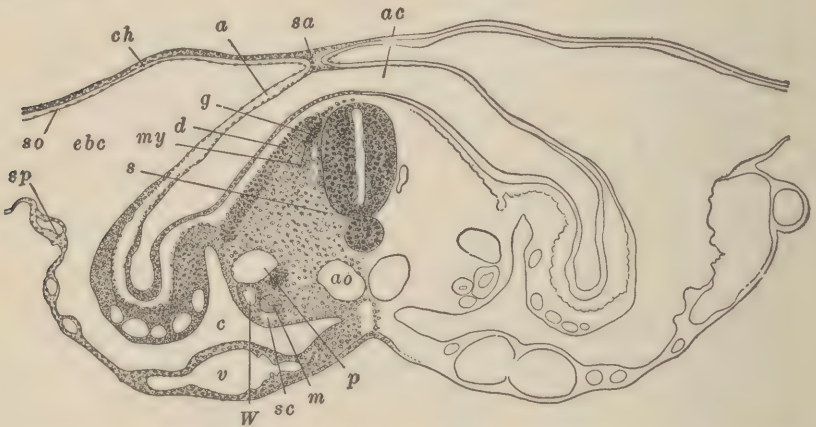
Fig. 167. — Transverse section of Chick embryo with thirty-five pairs of somites (about seventy-two hours), passing through the region of the twenty-third somite. From Kellicott (Chordate Development). After Lillie.

a. Amnion.　*ac.* Amnionic cavity.　*ao.* Dorsal aorta.　*c.* Embryonic cœlom.　*ch.* Chorion.　*d.* Dermatome.　*ebc.* exocœlom.　*g.* Rudiment of spinal ganglion.　*m.* Mesonephric tubule.　*my.* Myotome.　*p.* Posterior cardinal vein.　*s.* Sclerotome.　*sa.* Seroamnionic connection.　*sc.* Sub cardinal vein.　*so.* Somatic mesoderm.　*sp.* Splanchnic mesoderm.　*v.* Vitelline artery.　*W.* Wolffian duct.

The Amniotic Fluid. — Shortly after the completion of the amniotic cavity, fluid begins to accumulate within it. Thus the embryo is soon practically surrounded by a liquid cushion which protects it from pressure by its membranes and rigid shell. This is the *amniotic fluid*. Presently, about the fifth day muscle fibers develop in the mesoderm of the amnion and begin to send waves of contraction over it. This causes a gentle rocking of the embryo, and is apparently instrumental in preventing its adhesion to the various embryonic membranes. It may also help to obviate the stagnation of blood in the vessels, a condition which might tend to occur on account of the pressure from the growing organs.

THE SOMATIC UMBILICUS, THE YOLK-STALK, AND THE YOLK-SAC

Though they are not a part of the amnion, it seems best to include in connection with its description an account of these structures which, to some extent, develop with it.

The Somatic Umbilicus. — During the formation of the amnion, the gradual separation of the embryo from the yolk has been progressing. This has been accomplished by the steady in-pushing of the ventral portions of the head, tail, and lateral folds (*limiting sulci*) beneath the body of the growing Chick. The result is that by the time the amnion is completed, these folds have approached one another quite closely, though without coming into contact. In this manner they give rise to a short, thick, hollow stalk which connects the embryo with the yolk-sac and its extra-embryonic membranes. The outermost wall of this stalk is continuous with that of the amnion, and is, therefore, composed of ectoderm and somatic mesoderm: for this reason, this outer wall is referred to as the *somatic umbilicus* (Fig. 168).

The Yolk-Stalk. — Within this wall and surrounding the inner wall of the stalk, is a space continuous externally with the extra-embryonic cœlom and internally with the cœlom of the embryo itself. Finally, the inner wall of the stalk consists of splanchnic mesoderm and endoderm. It is known as the *yolk-stalk*, but is really merely an inner tube of the somatic umbilicus separated from it by cœlomic space.

The Yolk-Sac. — The wall of the yolk-stalk is coëxtensive within the embryo with the wall of the gut, and externally with the layer of endoderm and the splanchnic mesoderm which overlies the yolk. This layer is continually growing out around the yolk, and at its outermost border; i.e., the region of the zone of junction, the endodermal portion of it becomes continuous with the chorion which overlies it. Thus by means of the extension of these layers the yolk is gradually enclosed in a covering, whose inner layer of splanchnic mesoderm and endoderm constitutes the *yolk-sac,* attached to the embryo by means of the yolk-stalk. Upon the ninth day of incubation this sac has become virtually complete, save at a point on the side of the yolk postero-ventral to the body of the Chick, where an opening remains, known as the *yolk-sac umbilicus*. This opening, however, is finally closed about the seventeenth day by a solid mass of tissue. It may be recalled in this connection that the rim of the

blastoderm which has thus overgrown the yolk, was previously ho-
mologized with the lip of a very extended blastopore, the true blasto-
pore (primitive streak) having been separated from the remainder of
the rim during gastrulation.   Hence upon this basis it is possible to
consider the uncovered yolk mass as a sort of very large secondary,
or *yolk blastopore*, the latter term being really only another name
for the yolk-sac umbilicus.   A somewhat similar separate blastopore,
it may be noted, also occurs in the development of the Elasmobranchs

Figs. 168, 169, 170. — Diagrams of the relations of the extra-embryonic membranes in
the Chick. Figures and description from Lillie (Development of the Chick). The ectoderm
and endoderm are represented by plain lines; the mesoderm by a cross-hatched line
or band. The yolk-sac is represented by broken parallel lines. In Fig. 168 the allantois
is represented as a sac. In Figs. 169 and 170, where it is supposed to be seen in section,
its cavity is represented by unbroken parallel lines. The stalk of the allantois is exagger-
ated in all the diagrams to bring out its connection with the embryo.
    Fig. 168. — Fourth day of incubation. The embryo is surrounded by the amnion which
arises from the somatic umbilicus, *Umb.*, in front and behind; the sero-amniotic con-
nection, *S.am.*, is represented above the tail of the embryo; it consists at this time of
a fusion of the ectoderm of the amnion and chorion. The allantois, *All.*, is represented
as a sac, the stalk of which enters the umbilicus behind the yolk-stalk; the allantois
lies in the extra-embryonic body-cavity (exocœlom), and its mesodermal layer is fused
with the corresponding layer of the chorion above the embryo.   The septa of the yolk-
sac, *Y.S.S.*, are represented at an early stage.   The splitting of the mesoderm has
progressed beyond the equator of the yolk-sac, and the undivided portion is slightly
thickened to form the beginning of the connective-tissue ring that surrounds the yolk-
sac umbilicus. The ectoderm and endoderm meet in the zone of junction, beyond
which the ectoderm is continued a short distance. The vitelline membrane, *V.M.*,
is ruptured, but still covers the yolk in the neighborhood of the yolk-sac umbilicus.
The albumen is not represented in this figure. (For complete explanation of lettering
see Fig. 170.)

(i.e., the cartilaginous or non-bony fishes) in which the term yolk blastopore is regularly applied to it.

On the basis of this description, it is clear that beyond the boundaries of the amnion the chorion is really nothing more than the uppermost layer of the blastoderm. It is to be noted, however, that this upper layer consisting of ectoderm and somatic mesoderm is soon separated from the lower layer composed of splanchnic mesoderm and endoderm by the extra-embryonic cœlom. Furthermore, this space presently becomes occupied by another extra-embryonic organ (allantois), to be described below. Finally it must also be mentioned that early in its development, the lower layer, just indicated; i.e., the real yolk-sac layer, consisting of endoderm and splanchnic mesoderm, becomes covered internally with deep folds, the *yolk-sac septa,* which gradually press downward into the yolk. These septa in common with the remainder of the yolk-sac endoderm in the area vasculosa, contain glandular and absorbing cells which digest the yolk *in situ* before passing it into the blood vessels. Thus though a slight lumen exists in the yolk-stalk connecting the inside of the yolk-sac with the enteric canal, no yolk appears to pass into the embryo through this lumen.

The Allantois

Another extremely important extra-embryonic organ possessed in some degree by all Amniota is the *allantois,* and it will be found convenient to consider its entire history also at this time.

Its Early Development. — The allantois starts in the form of an out-pushing from the ventral wall of the hind-gut (Fig. 171). This is scarcely visible before the beginning of the third day, and was, therefore, not referred to in the foregoing description of the alimentary tract. This out-pushing naturally involves the endoderm and the mesodermal ventral mesentery which occurs in this region. Thus the sac which is presently formed possesses an inner endodermal and an outer mesodermal layer. By the fourth day the allantois has pushed out through the cœlomic space between the somatic umbilicus and the yolk-stalk, and is beginning to spread out in the extra-embryonic cœlom (Fig. 168). The narrow neck of the organ which then connects the outer sac-like portion with the gut is known as the *allantoic stalk.* Along this stalk pass the two *allantoic arteries* (later only one), and the single *allantoic vein,* to end in abundant ramifications over the surface of the sac. The allantois now grows rapidly, and within a couple of

days has entirely covered the amnion, occupying the space between that organ and the chorion. As it does so its mesoderm virtually fuses with the mesoderm of both chorion and amnion (Figs. 169 and 170). In this manner, the above ramifications of the blood vessels are brought very near to the shell, through which an exchange of gases is possible. Thus the allantois serves as an organ of respiration for the Chick during embryonic life. Its cavity also acts as a receptacle for the waste products of metabolism, which are conveyed thither through the allantoic stalk from the region of the cloaca. It is thus to be noted
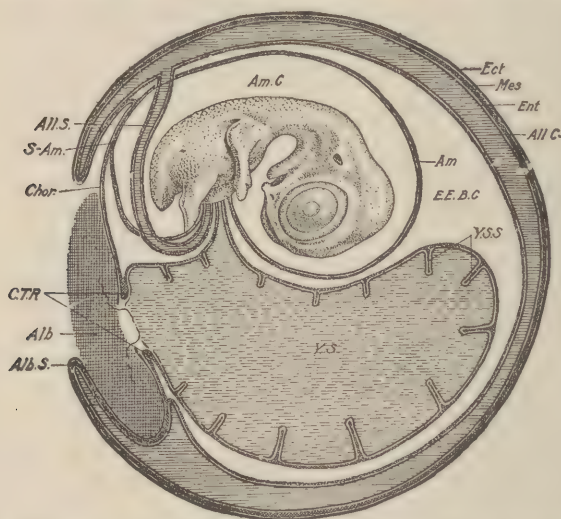
Fig. 169. — Ninth day of incubation. The yolk-sac umbilicus has become much narrowed; it is surrounded by the mesodermal connective-tissue ring, C.T.R., and by the free edges of the ectoderm and endoderm. The vitelline membrane still covers the yolk-sac umbilicus and is folded into the albumen. The allantois has expanded around the amnion and yolk-sac and its outer wall is fused with the chorion. It has pushed a fold of the chorion over the sero-amniotic connection, into which the meso-derm has penetrated, and thus forms the upper fold of the albumen-sac. The lower fold of the albumen-sac is likewise formed by a duplication of the chorion and allantois; it must be understood that lateral folds are forming also, so that the albumen is being surrounded from all sides. The stalk of the allantois is exaggerated so as to show its connection with the embryo; it is supposed to pass over the amnion, and not, of course, through the cavity of the latter. (For explanation of lettering see Fig. 170.)

that this organ is homologous not only in method of origin, but also partly in function with the urinary bladder of the Frog. The latter, however, of course never extends outside of the cœlomic cavity.

The Later Development of the Allantois and the Formation of the Albumen-Sac. — Meanwhile the albumen is becoming concentrated

on the side of the egg next to the yolk-sac umbilicus, and by the ninth or tenth day has become very much condensed. Concurrently the real yolk-sac layer, together with the chorion, has grown around the yolk so that the edges of the over-growth have more than kept in contact with the receding albumen. They have in fact thrust themselves in between it and the yolk, so that the albumen is bounded upon its inner side by a layer of chorion. At the same time, save postero-dorsally in the region of the sero-amniotic connection, the allantois has been following this over-growth of the yolk-sac layer
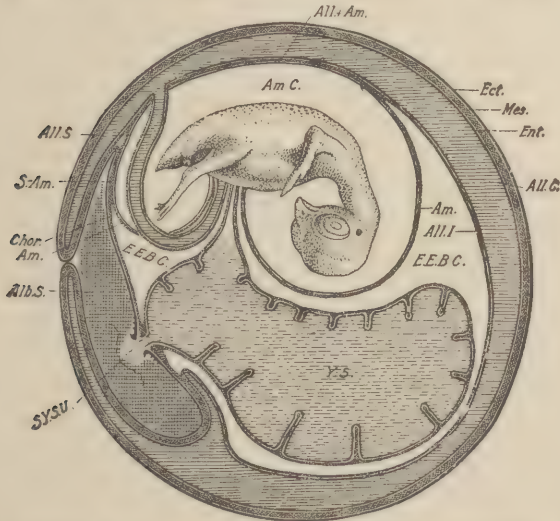
Fig. 170. — Twelfth day of incubation. The conditions represented in Fig. 169 are more advanced. The albumen-sac is closing; its connection with the cavity of the amnion by way of the sero-amniotic connection will be obvious. The inner wall of the allantois has fused extensively with the amnion. The umbilicus of the yolk-sac is much reduced, and some yolk protrudes into the albumen (sac of the yolk-sac umbilicus, transitory structure soon drawn into the yolk-sac proper).

Alb. Albumen. *Alb.S.* Albumen-sac. *All.* Allantois. *All.I.* Inner wall of allantois. *All.C.* Allantoic cavity. *All.S.* Allantoic stalk. *All.* + *Am.* Fusion of allantois and amnion. *Am.* Amnion. *Am.C.* Amniotic cavity. *Chor.* Chorion. *C.T.R.* Connective-tissue ring. *Ect.* Ectoderm. *E.E.B.C.* Exocœlom (extra-embryonic body-cavity). *Ent.* Endoderm. *Mes.* Mesoderm. *S.-Am.* Sero-amnionic connection. *S.Y.S.U.* Sac of the yolk-sac umbilicus. *Umb.* Umbilicus. (somatic). *V.M.* Vitelline membrane. *Y.S.* Yolk-sac. *Y.S.S.* Septa of yolk-sac.

and chorion; it lies between these two layers in the exocœlom, and its walls are fused respectively with the chorionic layer and that of the yolk-sac. Thus as the latter layers push in between the yolk and the albumen to close the yolk-sac umbilicus, they are accompanied, except

postero-dorsally, by the allantois. Ventro-laterally a fold of the
chorion presently pushes its way around the outside of the albumen
between it and the shell membrane. Here too, moreover, between
the two layers of the chorionic fold there follows an outer fold of the
allantois. Meanwhile in the postero-dorsal region, as already suggested,
the expansion of this organ is obstructed by the sero-amniotic connec-
tion. At this point therefore it pushes up over this connection,
carrying the chorion before it. Thus this dorsal fold, consisting of a
layer of chorion and allantoic wall, comes down between the albumen
and shell membrane to meet the similarly constituted ventro-lateral
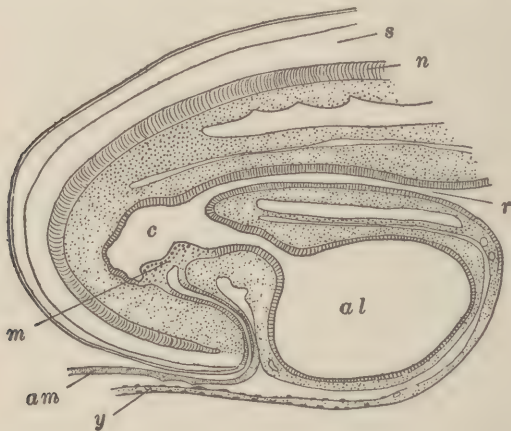
Fig. 171. — Median sagittal section through posterior end of four-day chick. From
Kellicott (Chordate Development). After Gasser (Maurer).
al. Allantois. *am*. Amnion (tail-fold). *c*. Cloaca. *m*. Cloacal membrane. *n*. Notochord.
r. Rectum. *s*. Spinal cord. *y*. Wall of yolk-sac (endoderm and splanchnic mesoderm).

folds already described. In this manner the mass of albumen at the
somatic umbilicus is virtually surrounded by a double layer of fused
chorionic and allantoic tissue, the *albumen-sac*. There is just one
region in the wall of the sac, however, where all of these layers are
not present. This is a small area on its internal dorsal side where the
allantois could not extend because of the sero-amniotic connection.
Here therefore the wall consists only of chorion, and at one point
of the connection itself (Figs. 169 and 170). Within this connection,
toward the close of embryonic life, a perforation occurs allowing some
of the albumen to pass into the amniotic cavity. The remainder of the
albumen is absorbed, and the albumen-sac together with the yolk-sac
is drawn within the embryo just previous to hatching. This latter

process is apparently considerably aided by the contraction of the muscular walls of the surrounding allantois, as well as by the muscles of the somatic umbilicus.

SUMMARY OF THE CONDITION AT THE END OF THE SECOND DAY OF INCUBATION

I. THE SOMITES

There are approximately twenty-seven somites, in which the *myotomes* and *cutis plates* have begun to differentiate, together with the mesenchymatous rudiment of the *sclerotome*.

II. THE FORE-GUT

In the fore-gut the *stomodæum* is formed, and in connection with it the *hypophysis* is beginning to appear. Four pairs of *visceral pouches* and five pairs of *arches* have begun to develop, and the first pair of pouches have acquired openings to the exterior. The rudiments of the *thyroid*, the *respiratory system*, and the *liver* are also present.

III. THE MID-GUT

This is but slightly developed, although the lateral folds are beginning to mark it off from the extra embryonic archenteron.

IV. THE HIND-GUT

The hind-gut has begun to form and its posterior end has fused with the ectoderm to form the *anal plate*. In connection with it there has also arisen the *ventral mesentery*.

V. THE CIRCULATORY SYSTEM

The Heart. — A bent *tubular heart* has been developed, lined by *endothelium* and covered with a *myocardium*. The regions of the *auricles*, the *ventricles*, and the *bulbus arteriosus* are indicated.

The Arteries. — The *dorsal aortæ*, the *ventral aorta*, the first three *aortic arches*, and the *vitelline arteries* have appeared.

The Veins. — The *anterior* and *posterior cardinals*, the *sinus venosus*, the *ductus venosus*, and the *ductus Cuvieri* have been developed. In connection with the latter the septa known as the *lateral mesocardia* have also been formed. Outside the embryo the *anterior vitelline veins* have arisen, and with them the rudiments of the *lateral vitelline veins*. The *sinus terminalis* has become complete.

VI. The Nervous System

The Flexures. — The majority of the important bendings or flexures, connected especially with the brain and spinal cord, but involving also the outer parts of the embryo, become evident on this day. Those appearing at this time are the *cranial flexure*, the *cervical flexure*, and the *lateral twist*.

The Brain and the Cranial Ganglia. — The *fore-brain*, *mid-brain* and *hind-brain* are now clearly indicated, and within the first main division certain parts are apparent, as follows: The outgrowth of the *optic stalks* is well advanced, and there may also be evident the rudiments of the *optic chiasma*, the *optic recess*, the *cerebral hemispheres*, the *infundibulum*, and some other minor structures. The roof of the mid-brain is becoming prominently arched.

The *cranial ganglia* of the V, VII and VIII, and IX and X nerves are visible, and the latter pair are beginning to separate.

The Spinal Cord and Ganglia. — The spinal cord has become thick-walled laterally, and has developed *ependymal* and *germinal* cells. The *neural crests* are segmenting to form the spinal *ganglia*.

VII. The Organs of Special Sense

The *optic vesicles* have become invaginated to form the *optic cups*, and the external ectoderm opposite each cup has invaginated in the process of forming a *lens*. In connection with the *ear*, the auditory portion of the ectoderm has become invaginated to form the *auditory sac*.

VIII. The Urinogenital System

Only the embryonic parts of the excretory portion of this system appear during the second day. These are the *pronephros*, including the *Wolffian duct*, and the rudiments of the *mesonephros*. These rudiments consist of concentrations of nephrogenous tissue, some of which are beginning to become vesicular in the formation of the *mesonephric tubules* and the *Malpighian bodies*.

IX. The Amnion

This extra-embryonic organ begins its development on the second day with the appearance of the *amniotic head fold*, the *amniotic lateral folds*, and sometimes an indication of the *amniotic tail fold*.

The complete development of the *amnion*, the *chorion*, the *allantois*, and the *yolk-sac* are described in this chapter.

THE CHICK: DEVELOPMENT DURING THE THIRD DAY OF INCUBATION

THE SOMITES

During the third day the number of pairs of somites increases to about thirty-six. The newer posterior somites, when first formed are in the same condition as were those which are now anterior, and are destined to go through the same process of development. Meanwhile, the more advanced anterior members of the series do not greatly change, except for further modification along the lines already indicated on the second day. These modifications are as follows:

THE DEVELOPMENT OF THE MUSCLE PLATES OR MYOTOMES, AND THE DERMATOME

Each myotome or muscle plate continues to grow down along the inside of its respective cutis plate, until in the most mature somites it reaches the ventral end of the cutis plate and fuses with it. In this manner a complete double layer of cells arises. In the inner layer or muscle plate thus formed, the cells or myoblasts presently begin to become spindle-shaped, reaching from the anterior to the posterior walls of each myotome. These are the rudiments of the future voluntary muscles. Somewhat later on the third day the outer or cutis plates of somites which have reached this stage begin to break up into mesenchyme, which wanders outward toward the ectodermal wall. There it eventually gives rise to the *dermis*.

THE SCLEROTOME

The sclerotomal mesenchyme continues to collect about the notochord and the sides of the nerve cord.

THE ALIMENTARY TRACT

The Fore-gut

The Oral Cavity. — During the third day, the oral plate breaks through, placing the stomodæum in direct communication with the pharynx (Fig. 181). The region in which the digestive tract opens to the exterior anteriorly is thus partly stomodæal and partly pharyngeal. It is called the *oral cavity*.

The Hypophysis. — This organ, it will be recalled, consists of a tongue of cells extending forward from the roof of the stomodæum toward the floor of the diencephalon in the vicinity of the infundibulum. At about the thirty somite stage it has nearly reached the
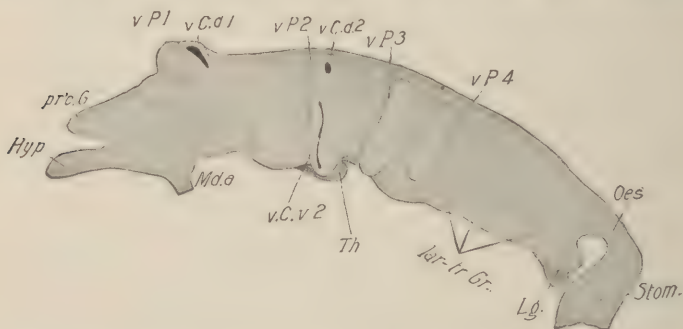
Fig. 172. — Reconstruction of the fore-gut of a Chick of 72 hours. From Lillie (Development of the Chick). After Kastschenko.

Hyp. Hypophysis. *lar-tr.Gr.* Laryngotracheal groove. *Lg.* Lung. *Md.a.* Mandibular arch. *Œs.* Œsophagus. *pr'o.G.* Preoral gut. *Stom.* Stomach. *Th.* Thyroid. *v.C.d.* 1, 2. Dorsal division of the first and second visceral clefts. *v.C.v.* 2. Ventral division of the second visceral cleft. *v.P.* 1, 2, 3, 4. First, second, third, and fourth visceral pouches.

latter organ (Fig. 181), and shortly its end begins to broaden out and become branched. Finally, near the end of the incubation period, the hypophysis has become a mass of tubular tissue well supplied with blood vessels. This glandular mass then loses all connection with the oral epithelium from which it arose, and becomes firmly attached to the infundibulum. It is often termed the *pituitary body*.

The Visceral Pouches and Arches.

The Pouches. — It will be remembered that during the second day four pairs of visceral pouches had appeared; the first three had reached

the ectoderm, and each member of the first pair had acquired a cleft opening to the outside. During the third day the first pair of pouches retain their openings, while each member of the second pair develops a short dorsal and a long ventral cleft, corresponding to the points of fusion between ectoderm and endoderm described in the preceding chapter. The members of the fourth pair of pouches now acquire connections with the ectoderm at their dorsal ends, but never develop any clefts (Fig. 172).

The Arches. — The visceral arches undergo no special change on the third day, except the development in some of them of the aortic blood vessels (arches) which will be described below.
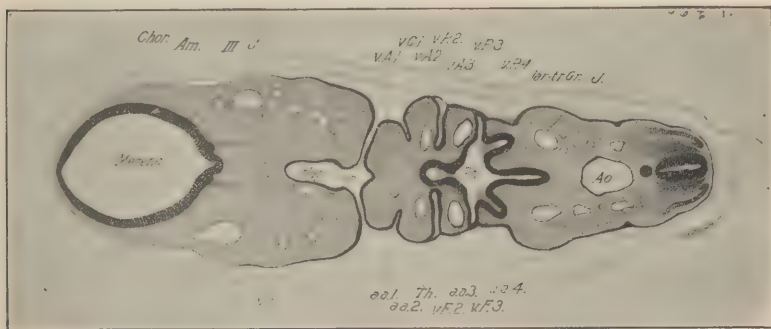
Fig. 173. — Frontal section through the pharynx of a 35 somite embryo. From Lillie (Development of the Chick).

a.a. 1, 2, 3, 4. First, second, third, and fourth aortic arches. *Hyp.* Hypophysis. *J.* Jugular vein. *lar-tr.Gr.* Laryngotracheal groove. *or.* Oral cavity *Ph.* Pharynx. *v.A.* 1, 2, 3. First, second and third visceral arches. *v.C.* 1. First visceral cleft. *v.F.* 2, 3. Second and third visceral furrows. *v.P.* 2, 3, 4. Second, third and fourth visceral pouches. *III.* Third cranial nerve.

The Thyroid. — During the third day, the rudiment of the thyroid which was last described as a slight depression in the floor of the pharynx, continues to evaginate. By means of this process, the end of the third day finds the above depression transformed into a wide-mouthed sac. Fig. 173 shows in cross section this and other structures indicated above.

The Laryngotracheal Groove and Lung Primordia. — At the end of the second day a shallow longitudinal groove had appeared in the floor of the pharynx just posterior to the visceral pouches, indicating the beginning of the respiratory system. This groove now becomes much

narrower and deeper, and is called the *laryngotracheal groove*. At its posterior extremity there also appears at this time a pair of postero-lateral out-pushings, the *primordia of the lungs* (Fig. 172).

The Œsophagus and the Stomach. — By the end of the third day the *œsophagus* is represented by an abrupt narrowing of the fore-gut immediately posterior to the pharynx. The narrowed portion leads into a slightly dilated region just anterior to the liver rudiment, and this dilation is the beginning of the *stomach;* i.e., the proventriculus and gizzard (see the fifth day).

The Liver. — At the end of the second day the liver was repre-sented by two anteriorly directed diverticula from the region of the

Fig. 174. — Reconstructions of the liver diverticula of the Chick. From Lillie (Devel-opment of the Chick). After Hammar.

A. On the third day of incubation; from the left side; the diverticula arise from the anterior intestinal portal.

B. Beginning of the fourth day; from the left side.

a.i.p. Anterior intestinal portal. *D.V.* Indicates position of ductus venosus. *g.b.* Gall bladder. *l.d.d.* (*cr.*). Dorsal or cranial liver diverticulum. *l.d.v.* (*caud.*). Ventral or caudal liver diverticulum. *pc.d.* Dorsal pancreas. *X.* Marks the depression in the floor of the duodenum from which the common bile duct is formed.

anterior intestinal portal; the more anterior of these had extended far enough forward to overlie slightly the point of union of the vitelline veins. During the third day, these diverticula grow somewhat further forward, the anterior member of the pair along the left dorsal side of the ductus venosus, and the posterior member along its right ventral side. Both now also branch profusely, the branches spreading around the ductus venosus and anastomosing freely with one another. At the same time capillaries from the ductus venosus begin to develop among

the interstices of these anastomosing branches; this is the beginning of the main body of the liver.

The Bile Ducts. — In the meantime, the intestinal portal has, of course, moved backward beyond the point of origin of the diverticula. This lengthens the gut and leaves these diverticula attached to its ventral side at their points of origin. The parts of the diverticula between the region of their anastomosis and the points of attachment to the gut are of the nature of short tubes, the rudiments of the future *bile ducts*. Presently the floor of the gut comprising the region where these ducts enter it becomes depressed and then drawn out so as to form a common duct into which the two original ducts empty. This common duct is called the *ductus choledochus,* and is a temporary structure (Fig. 174).

The Gall Bladder. — While the above processes have been going on, the *gall bladder* has arisen as a posterior evagination from the posterior liver diverticulum. As the latter then grows forward, its attachment to the gall bladder is drawn out to form the *cystic bile duct*.

The Pancreas. — This organ first appears on the third day as a thickening on the dorsal wall of the intestine about opposite the posterior liver diverticulum. The rudiment thus indicated gives rise to only about a third of the entire organ whose further development will be described as it occurs (Fig. 174).

THE MID-GUT

There is no great change in the mid-gut region during the third day except that it becomes more clearly marked off as the lateral folds continue to press in toward one another.

THE HIND-GUT

The Post-anal Gut. — It will be recalled that at the close of the second day the ectoderm had taken so slight a part in the tail fold that the anal plate retained a dorsal position. During the third day, however, the progress of the fold causes the anal plate to swing around the edge of the fold on to its ventral side. At the same time the posterior portion of the embryo lying just above the end of the gut starts to grow out and bend downward beyond the region of the anal plate. As it does so, it carries out with it a slender endodermal extension of

the hind-gut. This is a temporary structure known as the *post-anal gut* (Fig. 175).

The Allantois. — The most important structure to appear in connection with the hind-gut during early embryonic life is the allantois.
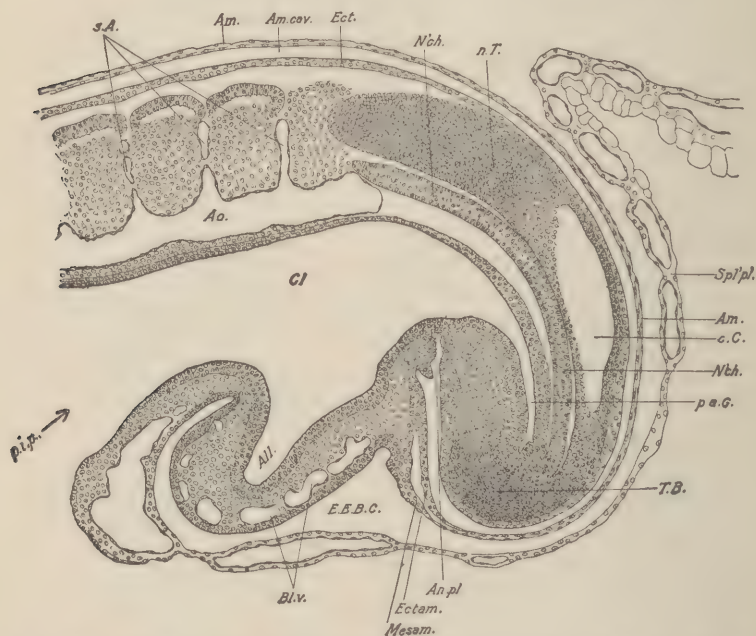
Fig. 175. — Sagittal section through the tail of an embryo of about 35 somites. From Lillie (Development of the Chick).

All. Allantois. *Am.* Amnion. *Am.cav.* Amnionic cavity. *An.pl.* Anal plate. *Ao.* Dorsal aorta. *Bl.v.* Blood-vessels in wall of allantois. *c.C.* Central canal of spinal cord. *Cl.* Cloaca. *Ect.* Ectoderm. *Ectam.* Ectoderm of amnion. *E.E.B.C.* Exocœlom. *Mesam.* Mesoderm of amnion. *N'ch.* Notochord. *n.T.* Nerve cord. *p'a.G.* Post-anal gut. *p.i.p.* Posterior intestinal portal. *s.A.* Segmental arteries, between the somites. *Spl'pl.* Splanchnopleure and yolk-sac entoderm. *T.B.* Tail bud.

The rudiment of this organ is usually indicated at about the beginning of the third day. The method of its development and its final structure have been described above (Fig. 171).

THE CIRCULATORY SYSTEM

THE HEART

There are no very marked changes in the form of the heart during the third day. The auricles become slightly more prominent and the bendings and constrictions already described are somewhat emphasized. Internally, sections reveal the fact that in the ventricular
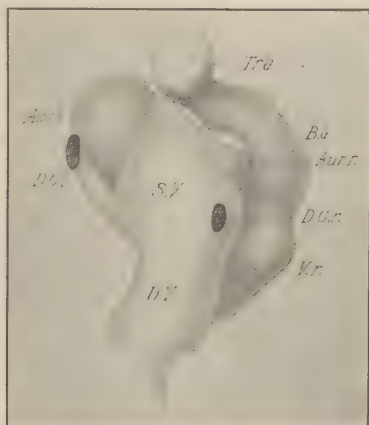
Fig. 176. — Heart of a Chick embryo of 72 hours, dissected out and drawn from the dorsal surface. From Lillie (Development of the Chick).

Aur.l. Left auricle. *Aur.r.* Right auricle. *B.a.* Bulbus arteriosus. *D.C.r.l.* Right and left ducts of Cuvier. *D.V.* Ductus venosus. *S.V.* Sinus venosus. *Tr.a.* Truncus arteriosus. *Vr.* Right limb of ventricle.

region the myocardium is becoming thickened and spongy. In the bulbus arteriosus, on the other hand, endothelial thickening has occurred, while the myocardium remains thin (Fig. 176).

THE EMBRYONIC BLOOD VESSELS

The Arteries.

The Dorsal Aortæ. — During the third day these vessels continue their development by beginning to form posterior to the point at which the vitelline arteries leave the body. These latter arteries thus become lateral branches of the dorsal aortæ, instead of their continuations, while the further posterior growth of these aortæ brings them
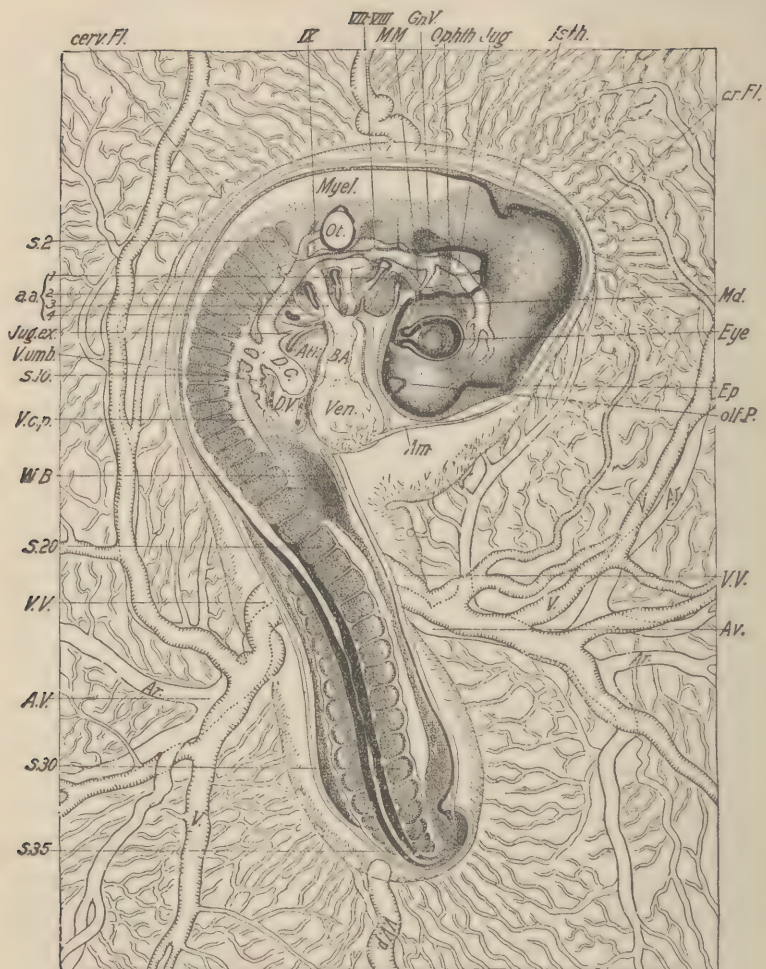
Fig. 177. — Chick embryo with adjacent portion of area vasculosa, with thirty-five pairs of somites (about seventy-two hours). Dorsal view. From Lillie (Development of the Chick).

a.a. 1, 2, 3, 4. First to fourth aortic arches. *Am.* Amnion. *Ar.* Branches of vitelline arteries. *Atr.* Atrium (Auricle). *A.V.* Vitelline artery. *B.A.* Bulbus arteriosus. *cerv. Fl.* Cervical flexure. *cr.Fl.* Cranial flexure. *D.C.* Ductus Cuvieri. *D.V.* Ductus venosus. *Ep.* Epiphysis. *Gn.V.* Ganglion of V cranial nerve. *Isth.* Isthmus. *Juq.* External jugular vein. *Md.* Mandibular arch. *M.M.* Maxillo-mandibular branch of V cranial nerve. *Myel.* Myelencephalon. *olf.P.* Olfactory pit. *Ophth.* Ophthalmic branch of V cranial nerve. *Ot.* otocyst. *s.2, s.10, s.20,* etc. Second, tenth, twentieth, etc., somites. *V.* Branches of the vitelline veins. *V.c.p.* Posterior cardinal vein. *V.umb.* Umbilical vein. *V.V.* Vitelline vein. *V.V.p.* Posterior vitelline vein. *W.B.* Wing-bud.

eventually to the extremity of the tail bud. Meanwhile anteriorly they have become fused, so that by the end of the second day a single aorta extends from just back of the aortic arches almost to the origin of the vitelline arteries. Finally during the fifth and sixth days the fusion of these vessels progresses into the tail region also, resulting in the formation of a single *caudal artery*. It will not be necessary, however, to trace these processes of growth and fusion in detail.

The Aortic Arches. — During the third day the main parts of the first aortic arches disappear. The dorsal portions, which are continuous posteriorly as the dorsal aortæ, however, presently extend ante-
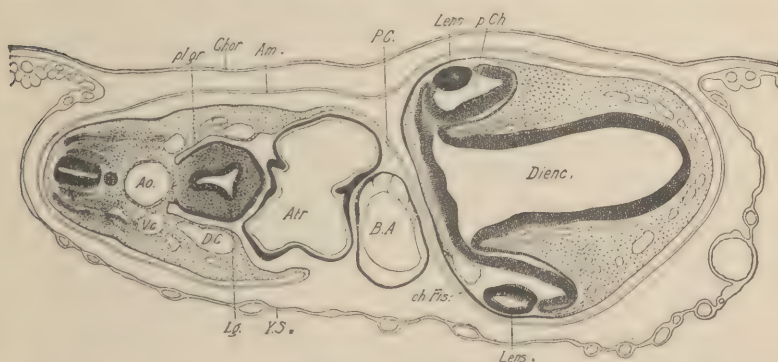
Fig. 178. — Transverse section, passing through the eyes and heart, of an embryo with about thirty-five pairs of somites (about seventy-two hours). Compare with figure 177. From Lillie (Development of the Chick).

Am. Amnion. *Ao.* Dorsal aorta. *Atr.* Atrium (Auricle). *B.A.* Bulbus arteriosus. *ch. Fis.* Choroid fissure. *Chor.* Chorion. *D.C.* Ductus Cuvieri. *Dienc.* Diencephalon. *Lg.* Rudiment of lung. *P.C.* Pericardial cavity. *p.Ch.* Posterior chamber. *pl.gr.* Pleural groove. *V.c.* Posterior cardinal vein. *Y.S.* Yolk-sac.

riorly as the *internal carotids*, while the ventral portions develop similarly as the *external carotids*. Meanwhile a *fourth aortic arch* arises in each of the fourth visceral arches.

The Pulmonary Arteries. — During the third day, these arteries appear as rudiments within the walls of the lungs.

The Veins.

The Cardinals and Jugulars. — During the third day, the anterior cardinals continue to branch considerably in the brain region and may now be know as the *internal jugulars*. At the same time a vessel from the floor of the pharynx joins each anterior cardinal (internal jugular) just at its point of union with the ductus Cuvieri. These new veins are

the *external jugulars* (Fig. 177). Late on the third day also a new pair of cardinals begins to develop. They arise from a series of anastomosing vessels on the ventral side of the mesonephros just lateral to the dorsal aorta, and are known as the *subcardinals*. They are scarcely apparent as definite vessels before the fourth day.

The Vitelline Veins. — Before leaving the body of the embryo, these veins become united by a short transverse vessel which passes over the
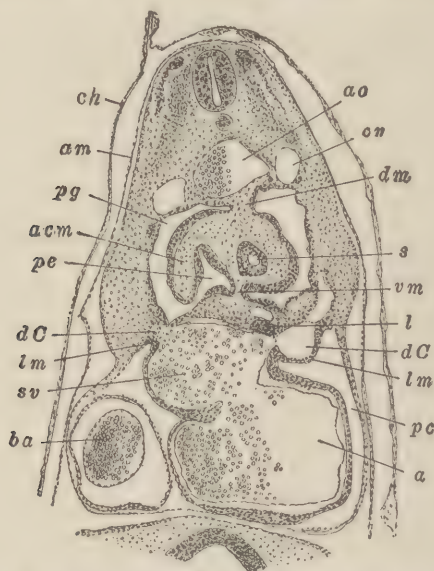
Fig. 179. — Part of a transverse section through the lateral mesocardia of a Chick with thirty-five pairs of somites (about seventy-two hours). From Kellicott (Chordate Development). After Lillie.

a. Auricle. *acm*. Accessory mesentery. *am*. Amnion. *ao*. Dorsal aorta. *ba*. Bulbus arteriosus. *ch*. Chorion. *cv*. Posterior cardinal vein. *dC*. Ductus Cuvieri. *dm*. Dorsal mesentery. *l*. Liver. *lm*. Lateral mesocardium. *pc*. Pericardial cavity. *pe*. Pulmo-enteric recess. *pg*. Pleural groove. *s*. Stomach. *sv*. Sinus venosus. *vm*. Ventral mesentery.

intestine just posterior to the dorsal pancreatic rudiment. In this manner, the intestine is surrounded by a venous ring. The anterior ventral part of this ring is formed by the posterior end of the ductus venosus. The lateral parts consist of the portions of the vitelline veins lying between the ductus venosus and the transverse vessel, and the posterior dorsal part is constituted of the transverse vessel itself (Fig. 188, *A, B*. See chapter XII). Meanwhile, as indicated

in the account of the liver, the portion of the ductus venosus which lies within that organ is beginning to give off capillaries among the branches of the liver diverticula.

The Umbilical Veins. — Early on the third day, a vein develops in the body wall on each side of the embryo, and opens anteriorly
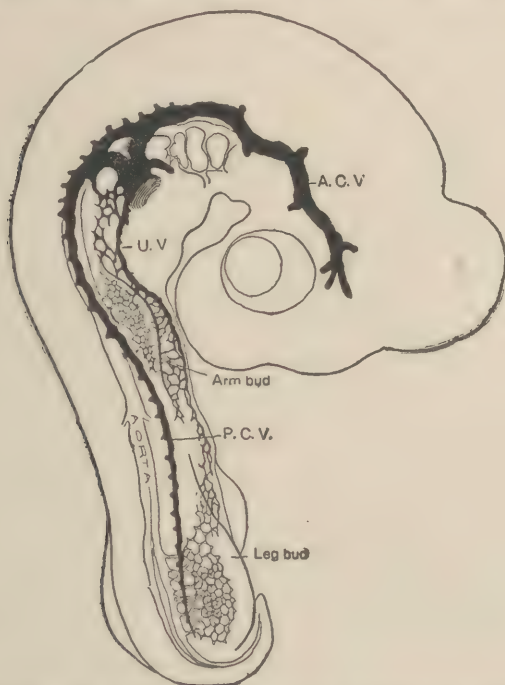
Fig. 180. — Injected Chick embryo of the third day, showing the arrangement of the cardinal veins and the formation of the umbilical vein from capillary networks. From Evans.

A.C.V. Anterior cardinal vein.   *P.C.V.* Posterior cardinal vein.   *U.V.* Umbilical vein.

into the ductus Cuvieri. These are the beginnings of the *umbilical veins*, although at this time they have no connection with the allantois (Fig. 180).

THE EXTRA-EMBRYONIC BLOOD VESSELS

The Arteries. — The vitelline arteries reach further out into the area vasculosa than during the second day, terminating near its border in a network of capillaries which empty into the sinus terminalis.

The Veins. — Posterior to the point where the anterior vitelline veins have fused, the right vein becomes greatly reduced. During this period, also, the lateral vitelline veins passing backward and outward along the margins of the anterior intestinal portal continue to form from the vascular network lying close to either side of the embryo. In this manner, they presently reach the region where the vitelline arteries turn rather directly outward into the area vasculosa, and at this point they also begin to pass outward just dorsal to the arteries. These veins never extend all the way to the sinus terminalis, but branch widely in the more central part of the vascular area. They receive blood from the terminalis, however, through several *intermediate veins* (venous trunks), which cross the outer network of arterial capillaries to reach them. Before the end of the third day, one other new extra-embryonic vessel starts to appear, the *posterior vitelline vein.* At this time it is scarcely more than a mass of capillaries, but very shortly begins to become distinct. It runs forward from the posterior side of the sinus terminalis, and empties into the left lateral vitelline vein near its base (Fig. 160).

THE NERVOUS SYSTEM

The Flexures

During the third day, the two flexures, cranial and cervical, both become more pronounced. The former flexure, together with the growth of the brain, results in bringing the prosencephalon even more markedly beneath the anterior end of the notochord. Indeed, these processes have gone so far that the morphologically anterior end of the brain or telencephalon, extends posteriorly until it almost touches the bulbus arteriosus of the heart. At the same time that these flexures have been increasing, the lateral twist of the body has been gradually progressing posteriorly from about the thirteenth somite to the twenty-first (Fig. 177).

The Fore-brain or Prosencephalon

The Telencephalon. — The indentation which marks the velum transversum becomes much more prominent, while the rudiments of the cerebral hemispheres grow in size and their walls increase in thickness.

In about the center of the lamina terminalis, a thickening appears called the *torus transversus*. It corresponds to the similarly named structure in the Frog, and as in that case it represents the rudiment of the future *anterior commissure*.

The Diencephalon. — The more anterior (ventral) portion of the diencephalon is now sometimes distinguished as the *parencephalon*, and the posterior (dorsal) portion as the *synencephalon* (Fig. 181). Between them is a slight constriction, while the parencephalon is bounded

Fig. 181. — Optical longitudinal section of the head of an embryo of 30s.   The heart is represented entire. From Lillie (Development of the Chick).

Atr. Atrium (auricles). *B.a.* Bulbus arteriosus. *D.v.* Ductus venosus. *Isth*. Isthmus. *Lg*. Laryngotracheal groove. *Oes*. Œsophagus. *or.pl*. Oral plate, which has begun to rupture. *Parenc*. Parencephalon. *Ph*. Pharynx. *Stom*. Stomach. *Synenc*. Synencephalon. *Th*. Thyroid. *S.v.* Sinus venosus. *Ven.R*. Right ventricle. Other abbreviations as before.

below by the marked indentation of the velum transversum. Thus the roof of the parencephalic region constitutes a relatively raised area from which the *epiphysis* begins to develop at the close of the day as a small out-pushing. Upon the floor of the diencephalon, the optic recess, the region of the optic chiasma, and the infundibulum all become more pronounced than they were at the end of the second day.

The Mesencephalon

The roof of the mid-brain grows rapidly and becomes prominently arched, while its walls increase uniformly in thickness. This arching of the mid-brain causes the boundary between it and the roof of the diencephalon to appear gradually more constricted. Likewise posteriorly at the connection between mid- and hind-brain, a slight constriction in the roof and lateral walls, indicated during the second day, also becomes very pronounced. This latter constricted region is henceforth known as the *isthmus*.

The Rhombencephalon

The Metencephalon. — After the isthmus has become established the thickening roof of the metencephalon consists largely of the wall forming the posterior side of the constriction. By the end of the day, the lateral walls of the metencephalon have also begun to thicken.

The Myelencephalon. — The roof of the myelencephalon remains thin, while its ventro-lateral walls have started to thicken somewhat.

The Spinal Cord. — At the end of the second day, the walls of the spinal cord were seen to consist chiefly of ependymal supporting cells and germinal cells. During the third day, the latter continue to multiply, and their descendants migrate out somewhat from their position near the central canal. In their new location, they presently become transformed either into neuroblasts; i.e., primitive nerve cells, or into primordial glia cells. The nerve cells even at this time have begun to send out the axones and dendrites typical of the adult neurones. The central parts of these neurones together with glia cells eventually come to constitute the *gray matter* of the cord, while the axones form its *white matter*.

As regards the final condition of the cord, the following may be said: Internally, the central canal is obliterated, save for a small ventral portion lined by the inner ciliated ends of the ependymal cells. Surrounding this and filling the central part of the cord is the gray matter with dorso-lateral and ventro-lateral extensions or horns reaching out into the white substance. Externally, there develops along both the dorsal and ventral sides a median longitudinal fissure. These fissures are formed mainly as a result of the enlarge-

ment of the lateral regions through the accumulation of the nerve fibers within them.

The Spinal Nerves. — The spinal nerves are of two kinds, sometimes described as constituting two systems; i.e., the *somatic nerves*, and the *splanchnic* or *sympathetic nerves;* both varieties start to develop during the third day. We shall consider the somatic nerves first.

1. The Somatic Nerves. — From bipolar nerve cells within each spinal ganglion one bundle of fibers (dorsal root) grows into the spinal cord, and another outward in a ventro-lateral direction. Together these constitute the *afferent* or sensory nerve fibers. At the same time from the ventro-lateral side of the nerve cord beneath each spinal ganglion, fibers (ventral root) are growing out from nerve cells located within the cord. These are *efferent* or *motor* fibers which mingle with those of the respective outgrowing afferent bundle just at the point where the latter leave their ganglion. The mixed fibers thus form the *common trunk* of a somatic spinal nerve. This trunk then divides again into a dorsal and ventral part, each part containing fibers of both the above types.   The
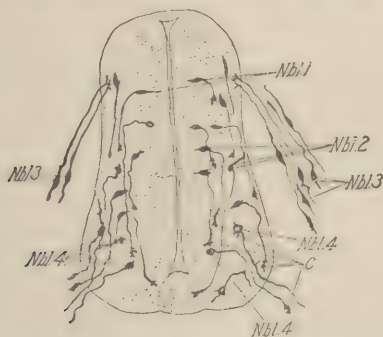
Fig. 182. — Transverse section through the spinal cord and ganglion of a Chick about the end of the third day; prepared by the method of Golgi. From Lillie (Development of the Chick). After Ramon y Cajal.

c. Cones of growth at the ends of growing nerve fibers. *Nbl.* **1** and **2.** Neuroblasts of the lateral wall. *Nbl.* **3.** Neuroblasts of the spinal ganglion. *Nbl.* **4.** Neuroblasts of the ventral horn (motor neuroblasts).

condition thus indicated is approximately the stage reached in the development of the somatic nerves at the end of the third day or early on the fourth (Fig. 182. Common trunk not shown).

Inasmuch as it will not be profitable in a work of this scope to follow further the detailed development of the somatic spinal nerves from day to day, their future arrangement will be summed up at this

time, as follows: The fibers of the divided trunks increase in number and at the same time grow outward. Hence, they almost immediately come into contact with the muscular and dermal plates, which are the rudiments of the future voluntary musculature and dermis of the Chick. Thus nervous connections are early established with these elements, and as the latter develop, the nerves (motor and sensory) develop with them.

II. The Splanchnic or Sympathetic Nerves. — At the end of the third day, cells are said to migrate downward from the spinal ganglia and form two cords of nervous tissue, one on either side of the dorsal surface of the aorta. These cords are the *primary sympathetic trunks.* Opposite each spinal ganglion, each of these trunks is slightly thickened, and from each thickening a cellular process runs upward to the ganglion. These thickenings may be termed the *primary sympathetic ganglia,* and the processes which connect them with the somatic system, the *primary rami communicantes* (Fig. 192. Chapter XII).

The Cranial Ganglia and Nerves. — The ganglia of the V, VII, VIII, IX and X nerves have already been described as appearing on the second day. During the third day, the V ganglion shifts its position of attachment to the brain somewhat, and its characteristic Y shape becomes more marked. The VII and VIII ganglionic mass also shifts to a more dorsal position. Otherwise the cranial ganglia show no marked alterations at this time (Fig. 177).

The Mixed Character of Certain Cranial Nerves. — In the Chick, as in the Frog, it is possible to distinguish the V, VII, IX, and X nerves as mixed; i.e., as containing both sensory and motor elements. In this respect they are of course not different from the spinal nerves, except as regards the point at which the two types of fibers become mingled. Thus in the region of the cord, the ventral or motor fibers of any nerve join the dorsal or sensory fibers of that nerve slightly *peripheral* to the dorsal ganglion. In the mixed cranial nerves, on the other hand, the two types of fibers issue from the brain very close together and mingle before entering the ganglion of the respective nerve. It may be further noted that though the ganglion of the VIII nerve is very closely associated at this time with that of the VII, its fibers are wholly sensory.

The III or Oculo-Motor Nerve. — Besides the mixed or wholly sensory nerves in the Chick, there are also, as in the Frog, certain cranial nerves which are purely motor and without any connection with the cranial ganglia. They take their origin from neuroblasts within the

brain itself, just as spinal motor nerves arise from neuroblasts within the spinal cord. The III or oculo-motor nerve arises in this manner from the median line of the ventral side of the mid-brain, at about sixty hours. Its history will be traced a few steps further in connection with the IV and VI nerves which arise on subsequent days.

THE ORGANS OF SPECIAL SENSE

THE EYE

The Optic Cup. — There are two main changes connected with the optic cup during the third day. The first change is the rapid increase in its size. Thus at the end of the second day the lens rudiment practically filled the cavity of the cup, and came in contact with its inner wall. At the end of seventy-two hours, on the other hand, the lens is entirely separated from the wall of the cup, and simply rests within its rim. The second change is the very evident thickening of the inner wall. The optic stalk is still markedly ventral as regards its point of attachment to the cup, the general region of the cup which surrounds this point being known as the *fundus* (Fig. 178).

The Lens. — The lens becomes detached from the superficial ectoderm during the third day, and forms a hollow ball, whose walls are at first of almost uniform thickness. Presently, however, the cells of the inner wall (i.e., the one next to the optic cup) begin to lengthen, in a direction at right angles to this wall, so that the latter is thereby thickened. By the end of the day this thickening has progressed to a considerable extent, the elongated cells which cause it being destined to form the lens fibers, which constitute the core of the lens.

THE EAR

At the end of the second day, the auditory pit had been transformed into the auditory sac, whose mouth was still partly open to the exterior. By virtue of the method of the closure of the pit, described in the previous chapter, the major part of the sac lies below the level of its external orifice. The connection of this orifice with the dorsal portion of the sac is then drawn out into a narrow tube, while the dorsal part of the sac itself is at the same time slightly constricted

away from the major ventral part. The former, or dorsal portion, is the rudiment of the *endolymphatic duct,* which presently grows upward somewhat so that its roof is slightly dorsal to the level at which the tube leading from it opens to the exterior (Fig. 183, *A*).

The Olfactory Organs

Early on the third day a small circular spot of ectoderm on each ventro-lateral side of the head somewhat in front of the eye becomes thickened, in consequence of a lengthening of its cells. These patches then begin to invaginate, and thus form the *olfactory pits* (Fig. 177). The thickened epithelium which lines them is the *olfactory epithelium,* and is said to consist of two types of cells, simple epithelial cells and germinal cells; the latter type later give rise to neuroblasts.
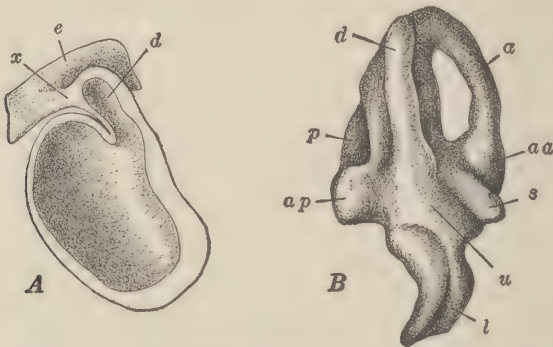
Fig. 183. — Two stages in the development of the auditory organ of the Chick. From Kellicott (Chordate Development). A. Hemisected model of the auditory sac (otocyst) just before its separation from the superficial ectoderm of the head. After Krause. B. Median view of a model of the left membranous labyrinth of an embryo of seven days and seventeen hours. After Röthig and Brugsch.

a. Anterior vertical semicircular canal. *aa.* Ampulla of anterior vertical semicircular canal. *ap.* Ampulla of posterior vertical semicircular canal. *d.* Ductus endolymphaticus. *e.* Superficial ectoderm of head. *l.* Lagena (cochlea). *p.* Rudiment of posterior vertical semicircular canal. *s.* Rudiment of saccule. *u.* Utricle. *x.* Connection between auditory sac and superficial ectoderm.

THE URINOGENITAL SYSTEM

During the third day, the pronephros degenerates, while the mesonephros continues to develop. Neither the metanephros nor the reproductive system appears during this period.

As regards the changes in the mesonephric region, it will be recalled that at the end of the second day the Wolffian or mesonephric portion of the pronephric duct was just beginning to acquire a lumen. Its backward-growing end, however, was still solid, and had not yet reached the cloaca. On the third day, this cellular rod connects with the cloaca, and by the end of the day a lumen has formed throughout its length. Concerning the mesonephros proper, at forty-eight hours the rudiments of the mesonephric tubules were forming in the neighbor-
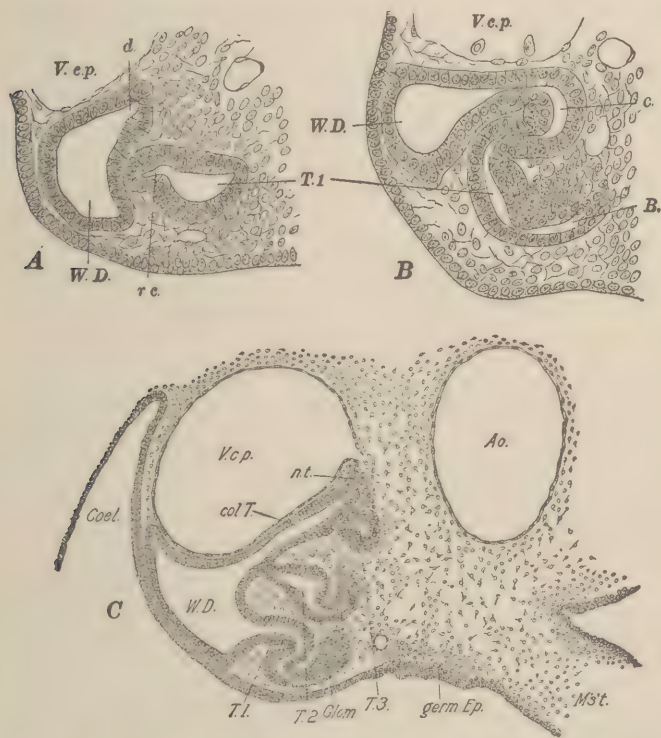
Fig. 184. — The development of the mesonephros. A.B. Transverse sections through the mesonephric tubules of the Duck embryo with forty-five pairs of somites. From Kellicott (Chordate Development). After Schreiner. C. Transverse section through the middle of the mesonephros of a Chick of ninety-six hours. From Lillie (Development of the Chick).

Ao. Dorsal aorta.   *B.* Rudiment of Bowman's capsule.   *c.* Conducting part of a primary tubule.   *cœl.* Cœlom.   *Col.T.* Collecting tubule.   *d.* Dorsal outgrowth of the Wolffian duct to form a collecting tubule (see fourth day).   *Glom.* Glomerulus. *germ.Ep.* Germinal epithelium.   *M's't.* Mesentery.   *n.t.* Nephrogenous tissue.   *rc.* Rudiment of conducting portion of primary tubule.   *T.* 1, 2, 3. Primary, secondary, and tertiary mesonephric tubules.   *V.c.p.* Posterior cardinal vein.   *W.D.* Wolffian duct.

hood of the twentieth somite or segment; i.e., in the most anterior region of the future organ. At that time, these rudiments, of which there were two or more to the somite, consisted merely of spherical condensations of the nephrotome, which were beginning to become vesicular. Now at the end of seventy-two hours, however, the vesicles in the most anterior mesonephric somites are giving rise to small, hollow evaginations in the direction of the Wolffian duct (Fig. 184, *A*). There is one evagination to each vesicle, and it is the part of the vesicle which is destined to form the actual mesonephric tubule. Indeed, just anterior to the twentieth somite or mesonephric region proper, some of the out-pushings have already become tubules and are connected through conducting portions with the Wolffian duct (Fig. 184, *B*). In this region also Malpighian bodies have appeared in connection with some of the tubules. These most anterior tubules and glomeruli, however, never become functional.

SUMMARY OF THE CONDITION AT THE END OF THE THIRD DAY OF INCUBATION

I. THE SOMITES

The number of pairs of somites has increased to thirty-six and in the more anterior pairs *dermatomes* and *myotomes* are completely developed. *Sclerotomal tissue* is still collecting about the notochord and the sides of the nerve cord.

II. THE ALIMENTARY TRACT

The Fore-gut. — The *oral plate* has broken through to complete the *oral cavity*, and the *hypophysis* reaches nearly to the *infundibulum*. The *second pair of visceral pouches* has acquired clefts, and the *fourth pair* has fused with the ectoderm. The *thyroid* depression has become a sac. The depression indicating the *respiratory system* has deepened into the *laryngotracheal groove*, and the *rudiments of the lungs* have appeared. The *œsophagus* and *stomach* are beginning to be defined. Finally, the *liver* diverticula have grown forward and anastomosed about the posterior part of the ductus venosus; the rudiment of the *gall-bladder is visible*, and the dorsal portion of the *pancreas* has appeared.

The Mid-gut. — It has become more clearly defined.

The Hind-gut. — The *anal plate* has been carried around to the ventral side by the progress of the tail fold, and at the same time the *post-anal gut* has been formed. The rudiment of the *allantois* has appeared.

III. THE CIRCULATORY SYSTEM

The Heart. — There are no external changes aside from an emphasis of curvatures and constrictions already present. In the *ventricular* region myocardial thickening has occurred, and in the *bulbus arteriosus* the same is true of the endothelium.

Embryonic Arteries. — Fusion of the *aortæ* has progressed. The *first pair of aortic arches* has disappeared, their dorsal and ventral remnants forming the *carotids*, while a *fourth pair of arches* has developed. The rudiments of the *pulmonary arteries* have arisen in the lungs.

Embryonic Veins. — The *anterior cardinals* have branched considerably in the brain region and are now known as the *internal jugulars* which receive the *external jugulars* just at the union of the former with the ductûs Cuvieri. The *ductus venosus* is beginning to develop capillaries among the branching liver diverticula. A new vessel passes over the intestine in the neighborhood of the pancreas and unites the vitelline veins to form a ring about the alimentary tract. A longitudinal vein has developed in each body wall; they are the *umbilical veins,* though at this time neither has acquired a connection with the allantois. The rudiments of the *subcardinal* veins may be visible on the ventral side of the mesonephros.

Extra Embryonic Arteries. — The *vitelline arteries* have pushed out into the area vasculosa until their branches nearly reach the sinus terminalis.

Extra Embryonic Veins. — The *right anterior vitelline* vein has almost disappeared; the *posterior* and *intermediate* vitelline veins have started to arise, and the *lateral vitelline* veins have developed further.

IV. THE NERVOUS SYSTEM

The Flexures and the Brain. — Both *flexures* have increased, while the *lateral twist* of the body has extended posteriorly. The *cerebral hemispheres* have grown somewhat, and the *epiphysis* has started to develop. The *optic chiasma,* the *optic recess,* and the

infundibulum have all become more clearly marked. The roof of the *mid-brain* is more prominently arched and the *isthmus* has appeared. There has also been thickening and thinning of the brain walls at various points.

The Spinal Cord and Spinal Nerves. — The *germinal cells* have changed their position and have begun to develop into *neurones* and *glia* cells. The *sensory* and *motor* nerve fibers issue respectively from the spinal ganglia and the ventral portion of the cord, the two types uniting to form the *common trunks* of the *somatic spinal nerves*. The *primary sympathetic trunks* and *ganglia* have appeared. The completion of the somatic portion of the spinal nervous system is described in this chapter.

The Cranial Ganglia and Nerves. — The ganglia have shifted their position slightly, and the third or *oculo-motor* nerves have appeared.

V. Organs of Special Sense

The Eye. — The *optic cup* has increased in size and its inner wall has thickened. The *lens* has become detached from the ectoderm, and its inner wall is also thickening.

The Ear. — The rudiment of the *endolymphatic* duct has appeared on the dorsal portion of the auditory sac.

The Olfactory Organs. — The *olfactory pits* have been formed, with walls consisting of epithelial and germinal cells.

VI. The Urinogenital System

The *pronephros* has begun to degenerate, while the *mesonephros* has started to develop tubules and glomeruli in its most anterior portion. The *Wolffian duct* has reached the cloaca and acquired a lumen throughout its length.

VII. The Amnion and Allantois

The folds of the *amnion* have approached one another above the posterior portion of the embryo and formed the *amniotic umbilicus*. The *allantois*, by about the middle of the day, has the appearance of a slight out-pushing from the hind-gut, and by the close of the day has extended well into the somatic umbilicus.

CHAPTER XII

THE CHICK: DEVELOPMENT DURING THE FOURTH DAY OF INCUBATION

THE SOMITES

THE COMPLETION OF THEIR FORMATION

By the end of the fourth day the number of somites has reached forty-two, and subsequent to this time ten more are added posteriorly. These last ten, however, later disappear, together with the four most anterior ones (head somites), which become fused with the skull. Thus at ninety-six hours the Chick possesses all the somites which take any part in the development of the adult Bird. The development of the myotomal and dermatomal elements progresses posteriorly in the manner already described.

THE ULTIMATE FATE OF MYOTOMES AND DERMATOMES

Although the ultimate disposition of these elements of the somites is not accomplished until some time later, it is not desirable to follow their development longer by one day periods. Regarding the dermatomes, or cutis plates, it has already been stated that their substance gradually moves out beneath the ectoderm, and ultimately forms the dermis. The muscle plates, as was also suggested above, go to form the muscles of the back, the body walls, and the limbs; in short, all the voluntary musculature of the Chick save that of the head. The latter develops from a part of the mesenchyme in that region. The involuntary muscles are likewise of mesenchymal origin.

THE SCLEROTOMES

During the third and fourth days the mesenchyme of the sclerotomes comes to occupy all spaces about the notochord and between the latter and the myotomes. Indeed, immediately around the notochord itself

it forms a thin continuous layer, the *perichordal sheath*. Further peripherally, however, a concentration of the mesenchyme in the cephalic and caudal portion of each sclerotome, as well as a slight division between these portions, has long made these parts distinguishable as such. Upon the fourth day, moreover, it begins to appear that upon either side of the notochord the cephalic half of each sclerotome is beginning to become fused with the caudal half of the one anterior to it, thereby establishing a new segmental arrangement (Fig. 185). From the method of their formation, it follows that the segments thus arising do not coincide with the myotomes; instead, they alternate with them just as they did in the Frog. In this manner, blocks of mesenchyme are being marked out on either side of the notochord; these are the rudiments of the right and left halves of the future *vertebræ*. Lastly, from the cephalic and caudal portion of each sclerotome, mesenchymatous tissue has now extended well upward around the sides of the nerve cord. This forms the rudiments of the *neural arches*, the cephalic arch of one sclerotome later fusing with the caudal of the next to form single arches corresponding to the vertebræ.

THE ALIMENTARY TRACT

THE REGION OF THE FORE-GUT

The Tongue. — The tongue appears on the fourth day as two papilliform outgrowths from the floor to the pharynx, one in front of and one behind the thyroid. These two rudiments then grow forward and fuse with one another. Eventually the structure thus constituted unites with a pair of lateral folds to form the tongue of the adult.

The Visceral Pouches and Arches.

The Pouches. — During the fourth day, the third pair of pouches acquire dorsal and ventral clefts like those of the second, while the clefts of the latter pouches and of the first (hyomandibulars) become closed. The second pouches then gradually disappear, whereas the dorsal portions of the first pair extend dorso-posteriorly toward the respective otocysts; here each eventually forms a part of the tubotympanic cavity (see fifth day).

The Arches. — The five pairs of arches reach their maximum development as such during the fourth day, and certain changes in their blood vessels take place; these changes will be described below.
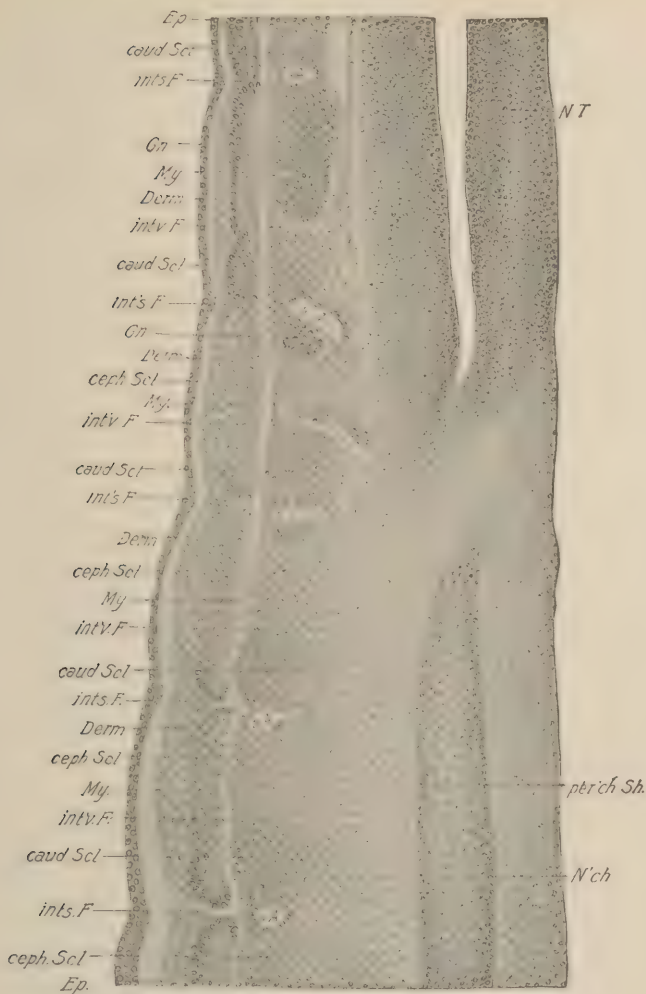
Fig. 185. — Frontal section through the base of the tail of a Chick embryo of **96 hours**. The anterior end of the section (above in the figure) is at a higher plane than the posterior end. From Lillie (Development of the Chick).

caud.Scl. Caudal division of the sclerotome. *ceph.Scl.* Cephalic division of the sclerotome. *Derm.* Dermatome. *Ep.* Epidermis. *Gn.* Ganglion. *int's.F.* Intersomitic fissure. *int'v.F.* Intervertebral fissure. *My.* Myotome. *N'ch.* Notochord. *N.T.* Neural tube. *per'ch.Sh.* Perichordal sheath. *s.A.* Segmental artery.

The Thyroid. — The thyroid sac at this time completely separates from the floor of the pharynx. Subsequently it becomes divided into two massive lobes which move backward and take up a position at the junction of the subclavian and the common carotid arteries.

The Respiratory Tract. — It will be recalled that at the end of the third day, the posterior part of the pharynx had deepened and narrowed to form the laryngotracheal groove, with the lung rudiments at its posterior extremity. During the fourth day, the posterior portion of this groove, including the lung diverticula, separates from the ventral part of the alimentary tract. The anterior portion of the new tube thus formed is the *larynx* which continues to open into the pharynx through a slit-like aperture, the *glottis*. The remainder of the tube is the *trachea,* which divides into the lung rudiments at its posterior end. This is the condition of the respiratory apparatus at the end of ninety-six hours.

The Œsophagus, the Stomach, and the Duodenum. — At the end of the third day, the fore-gut region posterior to the pharynx consisted of an elongated tube — the œsophagus, a slight dilation — the stomach, and finally another elongated region to which were attached the rudiments of the liver and pancreas. This last section of the fore-gut may from now on be termed the *duodenum.* During the fourth day the elongation of these parts continues, and also a certain curvature becomes evident. This latter process extends from the posterior region of the œsophagus to the end of the duodenum, and the direction of the bending is such that the convex side of the curve is toward the left.

The Liver. — It will be recalled that at the end of the third day the main body of this organ had formed an anastomosing network about the ductus venosus, and that it extended somewhat further forward on the left side than on the right. During the fourth day, this network increases, together with its interstitial blood vessels (Fig. 174, *B*). As this enlargement proceeds, it will be found that the larger part of the organ comes to lie more and more upon the right side of the body, in the hollow made by the bend of the stomach.

The Pancreas. — At the close of the third day, a thickening in the dorsal wall of the intestine opposite the posterior liver diverticulum was noted as the first rudiment of the pancreas. Upon the fourth day this thickening becomes a solid outgrowth, somewhat hollowed at its base. By the end of the day, two similar ventral rudiments may also be

visible as antero-lateral outgrowths from the common bile duct (the ductus choledochus). The subsequent union of these three elements will be described in the following chapter.

The Spleen. — Although this organ is not really a part of the digestive tract at all, it is convenient to describe its development at this point. During the fourth day a proliferation of cells occurs in the peritoneum at the base of the dorsal mesentery just above the dorsal pancreatic element. These cells become mingled with surrounding mesenchymal tissue, thus forming the main substance of the *spleen*. Subsequent development results in the formation of a considerable mass, filled with sinuses which communicate directly with the spleenic veins. Cells from the spleen are budded off into these spaces and pass into the circulation, where they become transformed into blood corpuscles.

THE REGION OF THE MID-GUT

For purposes of definition, the fore-gut region may be said to terminate at the end of the duodenum, and this point is marked approximately by the opening of the bile duct. The mid-gut, therefore, is the portion of the alimentary tract extending from the opening of this duct to the point at which the gut contained in the tail fold begins. It is difficult to define the latter point exactly at this time, except to say that since the tail fold never becomes very deep, it is relatively near the posterior end of the embryo, a short distance in front of the origin of the allantois. This boundary between the mid and hind-gut is marked later by the intestinal cæca (see Chapter XII).

During the third and fourth days the folding-in process has been going on rapidly in the region of the mid-gut, and thus at the end of ninety-six hours, the somatic umbilicus has become so much constricted that it is known as the *umbilical stalk*. Within it, as already described, are the allantoic stalk and the yolk-stalk. The former has always been small, and the latter has necessarily shared in the constriction of the umbilical walls. The result of these processes is obviously a mid-gut closed in at every point save the relatively narrow opening into the yolk-stalk; it is also a gut which still remains virtually straight. The section of alimentary tract which has thus been defined is destined to become the *small intestine* of the adult bird.

THE REGION OF THE HIND-GUT

The remainder of the digestive tract posterior to the small intestine is, by the above definition, the hind-gut, and constitutes the *large intestine* or *rectum*. This opens into a terminal chamber the *cloaca*. There is little to be said about the development of the rectum at this time, since it remains short, uncoiled, and without appendages.

The cloaca at ninety-six hours consists of a chamber into whose antero-dorsal wall there opens, as indicated, the rectum. Just back of the rectal orifice, the cloacal cavity also receives the Wolffian ducts. Antero-ventrally below the rectal opening is the aperture of the allantois, while just behind this on the ventral side of the chamber is the original anal plate, or, as it is now sometimes called, the *cloacal membrane* (Fig. 171). It consists, as will be recalled, of a fused plate of endoderm and ectoderm, and during embryonic life separates the cavity of the cloaca from the exterior. Posterior to these apertures and the cloacal membrane, the cloacal chamber shows a marked lateral compression.

THE CIRCULATORY SYSTEM

THE HEART

In order to understand the development of the heart during the fourth and subsequent days, it will be necessary for the reader to refer to the description of that organ at the end of the second day. Assuming that this description is clearly in mind, we may then continue the account of the development on the fourth day, as follows:

Changes in the Proportion of the Parts. — The entire loop, exclusive of the auricular region, but including the ventricular and bulbus portions, has gradually been expanding so that its parts have tended to approach one another. This has also resulted in a relative shortening of the two ascending limbs AB (ventricular) and DC (ventricular and bulbus). Indeed, so great has been the expansion of the ventral transverse section of the ventricle, BD, that these limbs have been almost obliterated (Fig. 186, B, C). What remains of the left posterior one (AB) is marked by a constriction just below the developing auricles. In the midst of this constriction is the opening

between the auricles and the entire ventricular region; it is the *auricular canal*.

Changes in the Relative Position of the Parts. — At the same time that these changes in shape and proportion have been occurring, changes in the relative positions of the parts are also progressing.
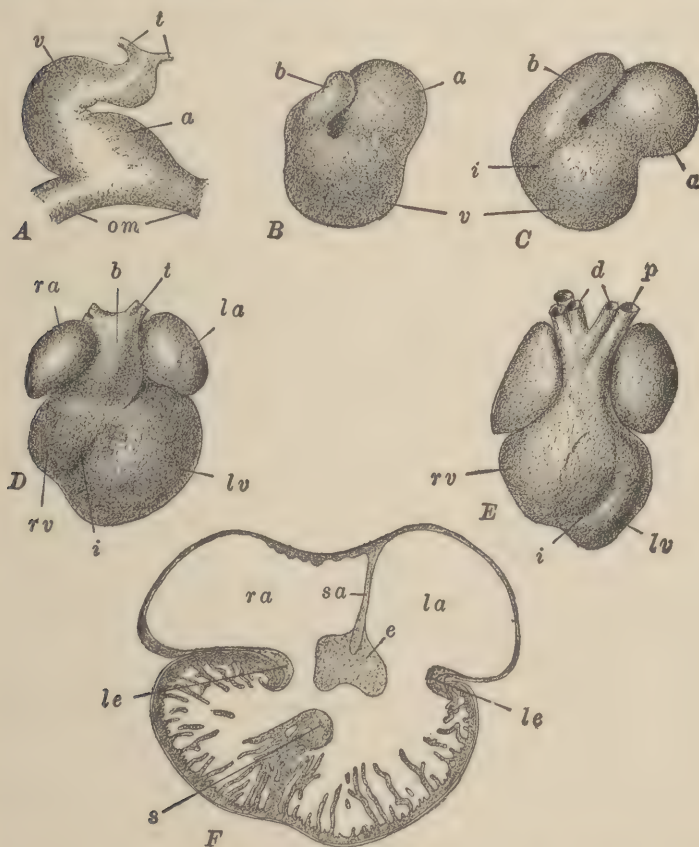
Fig. 186. — The development in the heart of the Chick. From Kellicott (Chordate Development). A, F, after Hochstetter. B-E, after Greil. A-E, ventral views of the heart. A. of a forty-hour embryo; B. of an embryo of **2.1** mm. head-length; C. of an embryo of **3.0** mm. head-length; D. of an embryo of **5.0** mm. head-length; E. of an embryo of **6.5** mm. head-length. F. Frontal section through the heart of an embryo of **9** mm. head-length.

a. Auricle. *b*. Bulbus. *d*. Roots of dorsal aorta. *e*. Median endothelial cushion (i.e., the cushion septum). *i*. Interventricular groove. *la*. Left auricle. *le*. Lateral endothelial cushion. *lv*. Left ventricle. *om*. Vitelline veins. *p*. Left pulmonary artery. *ra*. Right auricle. *rv*. Right ventricle. *s*. Interventricular septum. *sa*. Interauricular septum. *t*. Roots of aortic arches. *v*. Ventricle.

Of these there are three principal ones which may be indicated thus:
(1) The bulbus arteriosus is swinging toward the median line beneath
the auricles (Fig. 186, *D*). (2) The ventricular region is moving
backward behind the auricles and also somewhat toward the median
line, the region of the future apex pointing posteriorly. (3) To some
degree as a part of the latter movement, the posterior portion of the
auricles into which the sinus venosus opens is rotating forward. In
this manner, it is brought just over and then anterior to the auricular
canal, the latter remaining at a comparatively fixed point between the
ventricular and auricular regions. Though not completed during the
fourth day, these movements are well under way at this time. Their
progress, moreover, is sufficient to show that their tendency is to place
the parts of the heart more nearly in their adult positions; i.e., the
auricles anterior and dorsal, and the ventricles posterior and ventral.

Interior Changes Involving the Growth of Septa. — While the
above external alterations in the form of the heart have been going
on, there has begun internally the development of three important
septa: (1) From the wall of the auricular chamber, just to the left of
the opening of the sinus venosus, a septum starts to extend toward
the auricular canal; this is the *interauricular septum,* whose develop-
ment eventually separates the left auricle from the right. (2) At the
apex of the ventricle, the *interventricular septum* arises, and grows for-
ward. Now since the ventricular apex has become posterior to both
the auricular canal and the bulbus arteriosus, it is possible for the
forward extension of this septum to meet them both. This, it eventu-
ally does (see Chapter XII). (3) At the same time these septa
are developing, a third one is beginning to arise within the auricular
canal; it starts as two endothelial thickenings, one in the floor, and
the other in the roof of this canal. These are destined to grow
towards one another until they unite in the center of the auricular
aperture, thus dividing it into right and left parts. When completed,
this partition is known as the *cushion septum* (Fig. 186, *F*).

EMBRYONIC BLOOD VESSELS

The Arteries.

The Aortic Arches. — It will be recalled that during the third day,
the first pair of aortic arches disappeared, leaving the anterior ex-
tensions of the dorsal aortæ as the internal carotids. In a similar

manner, extensions from the bases of the first arches continue anteriorly as the external carotids. Upon the fourth day, the second aortic arches are likewise obliterated, and the two pairs of carotids continue posteriorly to the dorsal and ventral ends of the third pair of arches. At the same time two new pairs of aortic blood vessels develop in the vestigial fifth visceral arches behind the fourth and last pair of visceral pouches. These are the *fifth* and *sixth aortic arches* (Fig. 187, *A*). The fifth pair is small and quite transitory, being actually attached both dorsally and ventrally to the anterior sides of the sixth pair. Shortly after the sixth arches have thus arisen a small branch develops from about the middle of each and connects with the rudiments of the pulmonary arteries growing out from the
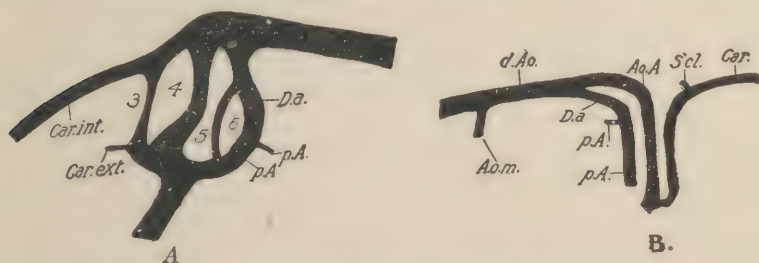
Fig. 187. — Aortic arches of the Chick. From Lillie (Development of the Chick). A. After Locy. A. Of the left side of a Chick of four and one-half days, from an injection. B. Reconstruction from sagittal sections of an eight day embryo (right side).

Ao.A. Arch of aorta (systemic arch). *A.o.m.* Vitelline artery. *Car.* Carotid artery. *Car.ext.* External carotid artery. *Car.int.* Internal carotid artery. *D.a.* Ductus arteriosus (Botalli). *d.Ao.* Dorsal aorta. *p.A.* Pulmonary artery. *S'cl.* Subclavian artery. 3–6. Third to sixth aortic arches (first to fourth " branchial " aortic arches).

lungs. In this manner the pulmonary arterial system is completed, though throughout embryonic life the branches just indicated remain small.

From this description, it is clear that only the ventral portions of the sixth arches take part in the formation of the pulmonary arteries. The dorsal portion of each arch, on the other hand, is known as the *ductus Botalli* or *ductus arteriosus*, which, as will be noted below, atrophies at the time of hatching.

The Subclavian Arteries. — During the fourth day, a pair of external swellings appears on the sides of the embryo somewhat posterior to the region of the heart. These swellings are the *anterior limb buds*. At the same time, the eighteenth segmental artery on each side gives

rise to a branch which extends out toward the respective bud. It is the *primary subclavian artery*.

The Sciatic Arteries. — Posteriorly, a pair of segmental arteries enlarge and grow out toward the *hind limb buds,* which have developed in a manner similar to the anterior pair. These vessels become the *sciatic arteries,* and as the legs develop they grow with and supply them.

The Umbilical Arteries. — During the fourth day, each sciatic artery gives off at its base a branch which extends into the allantois. These are the *umbilical* or *allantoic arteries.* Later (eighth day), the right member of this pair starts to disappear, while the left becomes a very important embryonic vessel, furnishing blood to the allantois. Indeed, so large does it become that the left sciatic seems for a time to be merely a branch from it.

The Renal Arteries and those of the Gonads. — Numerous branches from the dorsal aorta supply the mesonephros at this time, and later on a few of these persist as the *renal arteries.* Branches from the aorta also supply the reproductive organs as these develop.

The Veins.

The Vitelline Veins. — It will be recalled that at the close of the third day, the vitelline veins within the embryo had been united by a transverse vessel dorsal to the intestine, so that the latter was surrounded by a venous ring. Between this time and the close of the fourth day, further changes have taken place in this region, as follows: Very shortly after the transverse vessel has been formed the left side of the above ring disappears (Fig. 188, *C*). Later, as the anterior intestinal portal moves backward, the vitelline veins between the portal and the transverse vessel fuse with one another beneath the intestine. In this manner, a venous ring is again formed around the posterior extremity of the fore-gut, and in this case the right side presently begins to grow smaller. Anterior to the vitelline veins the ductus venosus continues to receive capillaries from the surrounding liver (Fig. 188, *D*).

The Cardinal Veins. — The anterior cardinals, as indicated in the previous chapter, have, by this time, reached a stage when they may be known as jugulars, while the posterior cardinals continue as previously described. The subcardinals which started to form on the third day become distinct vessels and presently acquire several direct

connections with the posterior cardinals lying on the dorso-lateral sides
of the mesonephros (Fig. 189).

The Inferior or Posterior Vena Cava. — This important vessel of
the adult Bird begins to develop at this time out of some of the capil-
laries in the dorsal part of the liver on the right side.   Slightly further
back it is also augmented by venous islands in a fold (the *caval fold*)
of one of the liver mesenteries.   These capillaries and venous islands
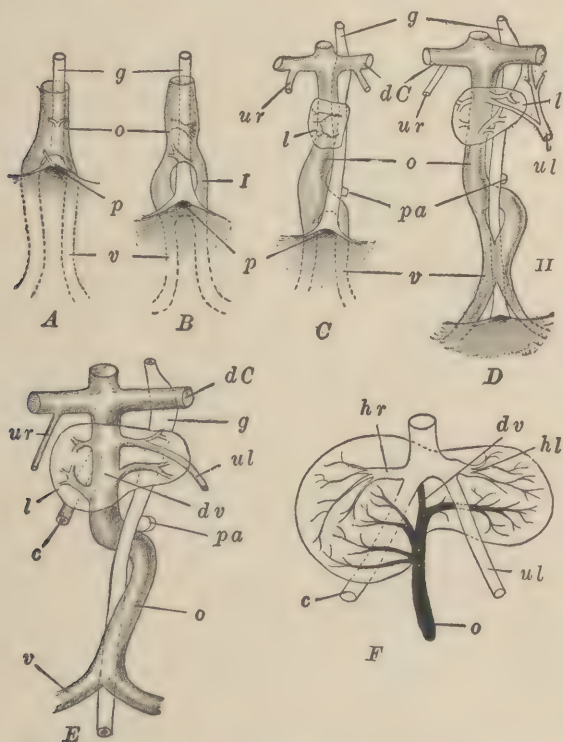
Fig. 188. — Diagrams illustrating the formation of the omphalomesenteric and um-
bilical veins, in the Chick, ventral view.   From Kellicott (Chordate Development).
After Hochstetter. A. At about fifty-eight hours. B. At about sixty-five hours. Veins
joined dorsal to the gut. C. At about seventy-five hours. Veins again separate. D.
At about eighty hours. Secondary union of veins around the gut. E. At about one
hundred hours. F. At about one hundred and thirty hours just before the disappearance
of the main portion of the ductus venosus within the liver.

c. Vena cava posterior (inferior). *dC.* Ductus Cuvieri. *dv.* Ductus venosus. *g.* Gut.
hl. Left hepatic vein.   *hr.* Right hepatic vein.   *l.* Liver.   *o.* Omphalomesenteric or
vitelline vein (the posterior continuation of the ductus venosus). *p.* Anterior intestinal
portal. *pa.* Rudiment of pancreas. *ul.* Left umbilical vein. *ur.* Right umbilical vein.
v. Vitelline vein. *I, II.* Primary and secondary venous rings around the gut.
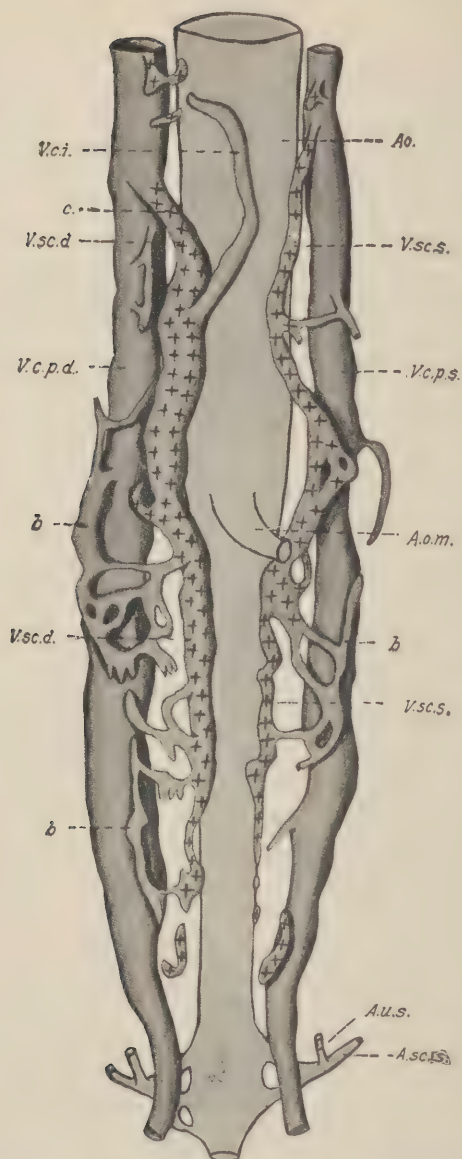
Fig. 189. — Reconstruction of the venous system of a Chick, 90 hours, ventral view. From Lillie (Development of the Chick). After Miller.

A.o.m. Omphalomesenteric (vitelline) artery. a.sc.s. Left sciatic artery. A.u.s. Left umbilical artery. b. Vessels enclosed within ventral side of mesonephros. c. One of the direct connections of subcardinal with posterior cardinal. V.c.p.d., s. Right and left posterior cardinal veins. V.c.i. Vena cava inferior. V.sc.d., s. Right and left subcardinal veins.

soon fuse together so as to form a definite vein which empties anteriorly into the ductus venosus (Fig. 188, *E*), and posteriorly establishes a connection with the right subcardinal (Fig. 189). Its subsequent development will be described in the following chapter.

The Umbilical Veins. — Upon the fourth day, the veins of the lateral body wall acquire connections with efferent vessels which have developed in the allantois, and at the same time, the right vein begins to disappear. The left vein on the other hand persists, but presently loses its anterior outlet into the ductus Cuvieri. At the same time, however, it develops new connections with the anterior half of the ductus venosus (Fig. 188, *D*, *E*). Through these, therefore, blood from the allantois flows quite directly into the latter vessel, without taking any extensive part in the hepatic portal circulation. Later, these connections with the ductus venosus fuse into one, which thus constitutes the anterior extremity of the single umbilical vein (Fig. 188, *F*). Eventually this vein acquires a median position in the embryo instead of its original lateral one. Subsequent to hatching, its proximal portion persists as a vein of the ventral body wall.

The Pulmonary Veins. — These vessels also develop at about this period in connection with the rudiments of the lungs, and presently become connected with the heart in the region of its left auricle.

Extra-Embryonic Blood Vessels

The Arteries. — During the fourth day the proximal portions of the vitelline arteries become fused with one another so as to leave the dorsal aorta as a single vessel. This fusion, however, occurs for only a relatively short distance, and never passes beyond the end of the umbilical stalk. From that point, the two main vessels continue to run out laterally, branching as they go, and terminating in a network of capillaries just inside the sinus terminalis. Subsequent development does not fundamentally alter the arterial plan except that as the septa of the splanchnopleure develop in the yolk sac, the arterial capillaries come to occupy the deeper portions of these septa.

The Veins. — By the end of this day the right anterior vitelline vein has disappeared, while the left anterior vitelline vein and the posterior vein, are well developed. The lateral vitelline veins have also become larger and more definite at the point where they extend outward in company with the arteries. Further out in the area

vasculosa, they continue to branch extensively, the branches connecting with the intermediate veins as already noted. By this time, however, these connections are so pronounced that the intermediate vessels appear merely as the finer endings of the lateral vitellines, uniting these veins with the sinus terminalis (Fig. 160). Subsequent to the tenth day, the anterior and posterior vitelline veins are gradually eliminated, the lateral veins persisting as the main efferent vessels of the yolk sac. After the tenth day, the sinus terminalis is no longer
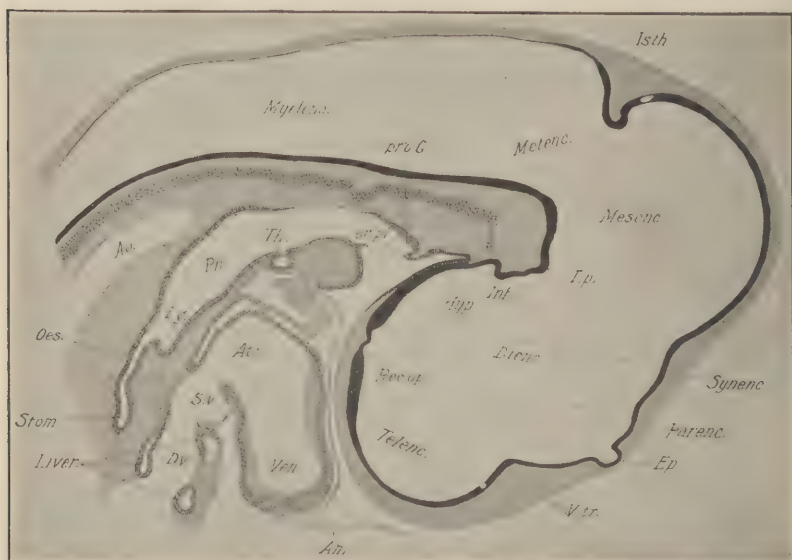
Fig. 189A. — Optical longitudinal section of the head of an embryo of 39s. From Lillie (Development of the Chick).

Atr. Atrium (auricles). *B.a.* Bulbus arteriosus. *D.v.* Ductus venosus. *Lg.* Laryngotracheal groove. *Œs.* Œsophagus. *or.pl.* Oral plate, which has now ruptured. *Parenc*. Parencephalon. *Ph.* Pharynx. *Stom.* Stomach. *Sunenc*. Synencephalon. *Th.* Thyroid. *S.v.* Sinus venosus. *Ven.* ventricle. Other abbreviations as before.

distinct, becoming obliterated by a mass of capillaries. These capillaries and the vessels with which they are connected, forming the area vasculosa, then continue to spread over the yolk in company with the yolk sac mesoderm. Thus, like the latter, they come at last virtually to surround it.

THE NERVOUS SYSTEM

This system, like the others, continues to develop throughout embryonic life. The differences observed in it between the fourth and fifth days, however, are not, in most respects, very great. Therefore, since it is not proposed to carry a detailed chronological description of any of the organs beyond the fifth day, we shall conclude the account of the nervous system in the present chapter.
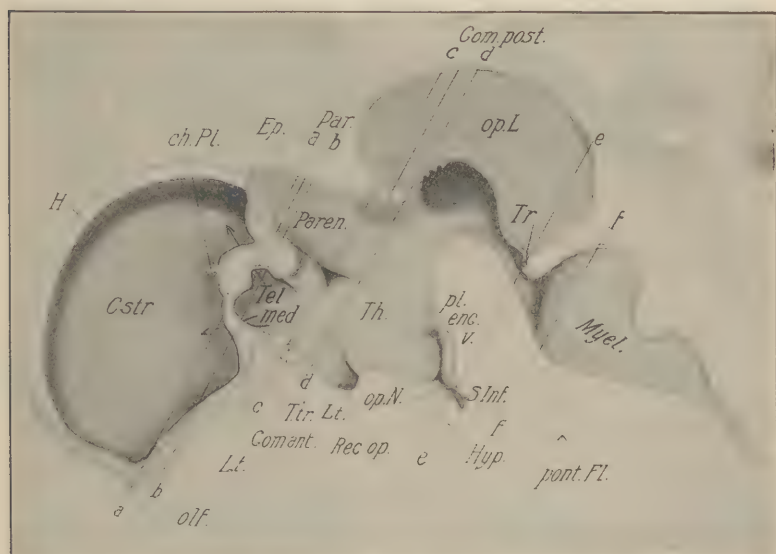
Fig. 190. — Dissection of the brain of an 8-day Chick. From Lillie (Development of the Chick). The arrows shown in the figure lie near the dorsal and ventral boundaries of the foramen of Monro.

ch.Pl. Choroid plexus (anterior). *Com.ant.* Anterior commissure. *Com.Post.* Posterior commissure. *C.str.* Corpus striatum. *Ep.* Epiphysis. *H.* Hemisphere. *Hyp.* Hypophysis. *L.t.* Lamina terminalis. *Myel.* Myelencephalon. *olf.* Olfactory nerve. *op.N.* Optic chiasma. *op.L.* Optic lobe. *Par.* Paraphysis. *Paren.* Parencephalon. *pl.enc.v.* Plica encephali ventralis. *pont.Fl.* Pontine flexure. *Rec.op.* Recessus opticus. *S.Inf.* Saccus infundibuli. *Tel.med.* Telencephalon medium. *Th.* Thalamus. *T.tr.* Torus transversus. *Tr.* Commissura trochlearis.

The lines a-a, b-b, c-c, d-d, e-e, f-f, represent the planes of sections not figured in this text.

THE FLEXURES

The cranial and cervical flexures continue to increase somewhat during the fourth day. A new flexure, also, is barely indicated by a slight ventral bulge in the floor of the myelencephalon. This is the

pontine flexure, and like the cranial flexure is destined to be permanent (Fig. 190). The lateral twist of the body, which at the end of the third day had reached approximately the twenty-first somite, is completed during the fourth day, so that the chick lies entirely on its left side. Subsequently the pontine flexure becomes somewhat more marked while the cervical flexure gradually disappears.

THE PROSENCEPHALON

The Telencephalon. — The cerebral hemispheres continue to increase in size during the fourth day, and their lateral walls in particular, are thickening to form the *corpora striata*. The other features already noted as characteristic of this portion of the brain have also increased in prominence. As regards subsequent development the

Fig. 191. — Median sagittal section through the brain of the Chick of twelve to thirteen days. From Von Kupffer (Hertwig's Handbuch, etc.)

c. Cerebellum. *ca*. Anterior commissure. *cd*. Notochord. *ch*. Habenular commissure. *ci*. Infundibular commissure. *ck*. Central canal of spinal cord. *cp*. Posterior commissure. *cpa*. Anterior pallial commissure. *cs*. Spinal commissure. *cv*. Cavum cerebelli. *cw*. Optic chiasma. *dr*. Epiphysial (pineal) gland. *dt*. Decussation of the trochlear (IV) nerve. *e*. Epiphysis. *e¹*. Paraphysis. *hm*. Cerebral hemisphere. *hy*. Hypophysis. *j*. Infundibulum. *le*. Ependymal lamina of the roof of the fourth ventricle. *lo*. Olfactory lobe. *lp*. Posterior lobe of cerebral hemisphere. *M*. Mesencephalon. *opt*. Optic chiasma. *pch*. Choroid plexus third ventricle. *pl*. Choroid plexus of fourth ventricle. *re*. Epiphysial recess. *ro*. Optic recess. *s*. Saccus infundibuli. *si*. Posterior intracephalic furrow. *tp*. Tuberculum posterius. *tp¹*. Tuberculum mammillare. *tr*. Torus transversus. *va*. Velum medullare anterius. *vi*. Median ventricle of telencephalon. *vp*. Velum medullare posterius.

cerebral hemispheres ultimately become one of the most noticeable portions of the brain, their backward growth causing them to overlap, and to conceal partially the large optic lobes. Their surface, however, never attains the complicated convolutions so characteristic of the Mammal. Anteriorly, beginning about the eighth day, small portions of these hemispheres become partially constricted away from the main posterior parts to form the *olfactory lobes*.

Concerning other parts of the telencephalon, as already indicated, the anterior commissure appears in the midst of the torus transversus. On the fifth day, also, an evagination develops at the antero-dorsal boundary of the lamina terminalis just between it and the velum transversum; it is the *paraphysis*. Above this body, the velum transversum eventually becomes folded to form the *anterior choroid plexus*.

The wall of this portion of the fore-brain, therefore, gives rise to the anterior commissure, the cerebral hemispheres, and most of the anterior choroid plexus. Its cavity forms the anterior part of the third ventricle into which the lateral ventricles of the hemispheres open through the foramina of Monro.

The Diencephalon. — In the roof of this region of the brain, the epiphysis shows no great change on the fourth day. Later, however, it grows out into a long narrow tube, whose end is dilated and possessed of numerous buds, the *epiphysial* or *pineal gland*. Just posterior to this organ at the boundary between the fore and mid-brains, the *posterior commissure* eventually becomes developed in the thickening which has marked this point from the first.

During the fourth day no striking development occurs in the lateral or ventral region of the diencephalon. Subsequently, however, the former region becomes greatly thickened as the *optic thalami*. On the ventral side, the fate of the infundibulum has already been described (see discussion of fore-gut, third day) while the *optic chiasma* comes to comprise a thick bundle of fibers from the optic nerves.

The floor of this posterior division of the fore-brain thus gives rise to the optic stalks, the optic chiasma and the infundibulum, while the optic thalami develop within the lateral walls. The roof forms a small portion of the anterior choroid plexus and the entire epiphysis; the cavity constitutes the posterior part of the third ventricle.

The Mesencephalon

There is nothing in particular to be said concerning the development of this region on the fourth day. Later we find that the growth and thickening of the dorso-lateral parts of the mid-brain greatly exceeds that of a narrow dorso-median strip, thus producing the two large *optic lobes,* which the median strip separates from one another by a fissure. Ventro-laterally, the sides and floor of the mid-brain also become thickened, constituting the *crura cerebri.* This thickening finally results in narrowing the central canal to form the *aqueduct of Sylvius* or *iter,* which connects the cavities of the third and fourth ventricles.

The Rhombencephalon

The Metencephalon. — The thickening which was noted in the roof of this region on the third day continues to increase, resulting finally in the production of a large median lobe, and two small lateral lobes united with it. The body thus formed extends backward somewhat so that it partially overhangs the myelencephalon. It is the *cerebellum.* About the ninth day, transverse fissures appear on the surface of this organ, which deepen as development proceeds. The ventro-lateral walls of the metencephalon, which have also been thickening, come eventually to form the *pons Varolii.*

The Myelencephalon. — It has already been stated that the roof of this region of the brain remains thin; it eventually forms the *choroid plexus* of the fourth ventricle. The ventral and ventro-lateral walls, however, showed signs of thickening on the third day. This tendency increases, and these walls finally constitute the *medulla oblongata.*

The Spinal Cord and Spinal Nerves

The description of the development of the cord and of the somatic spinal nerves was completed in chapter XI. The further development of the sympathetic system, however, remains to be described at this time.

The Completion of the Sympathetic System. — It will be recalled that at the end of the third day, the primary sympathetic system had

just been established. It consisted chiefly of two slender nerve cords and their ganglia lying just dorso-lateral to the dorsal aorta, and extending from the region of the vagus ganglion to the tail. On the fourth and fifth day, cells migrate from these cords and form aggregations beneath the aorta and in the mesentery of the intestines. On the sixth day also, the beginning of the *secondary* or *permanent sympathetic system* appears in the form of ganglia lying above the primary cords just median to the point where each somatic common trunk divides (Fig. 192). There are originally thirty pairs, one corresponding to the vagus ganglion and one to each pair of somatic ganglia as far
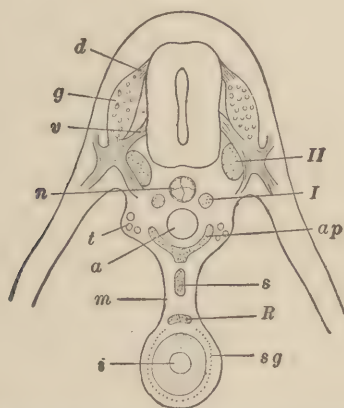
Fig. 192. — Diagram of the chief elements of the sympathetic nervous system of the Chick, in transverse section. From Kellicott (Chordate Development). After His. Jr.
a. Dorsal aorta. *ap*. Aortic plexus. *d*. Dorsal (afferent) root of spinal nerve. *g*. Spinal ganglion. *i*. Intestine. *m*. Mesentery. *n*. Notochord. *R*. Remak's ganglion. *s*. Splanchnic plexus. *sg*. Sympathetic elements in intestinal wall. *t*. Mesonephric tubules. *v*. Ventral (efferent) root of spinal nerve. *I*. Primary sympathetic cord. *II*. Secondary sympathetic cord. The rami communicantes are only partially shown.

back as the twenty-ninth. They are the *permanent* or true *sympathetic ganglia* and have originated like the primary system by the migration of neuroblasts from the dorsal spinal ganglia. Presently the sympathetic ganglia of each side become connected by longitudinal axones, thus forming the two *permanent sympathetic* cords. Other axones soon appear which connect each permanent sympathetic ganglion with the corresponding spinal ganglion of its respective side. These axones constitute the *permanent rami communicantes*, and represent both afferent and efferent fibers connecting the spinal cord with the sympathetic

system. The primary sympathetic cords now disappear as such, their cells going to form certain visceral plexuses and nerves which soon acquire connections with the ganglia of the secondary sympathetic system. The cardiac and other visceral plexuses arise meanwhile by cells which migrate from the tenth cranial ganglion.

The Cranial Ganglia and the Mixed Nerves.

Trigeminal Ganglion and Nerve. — It has already been stated that the ganglion of the V nerve has the form of an inverted Y. During the fourth day axones extend outward from the antero-dorsal branch of the Y, and pass anteriorly along the dorso-median walls of the optic vesicle. Eventually the nerve thus formed reaches the face and beak; it constitutes the *ophthalmic branch* of the fifth nerve. From the other branch of the Y, a second nerve extends toward the angle of the mouth; it is the *mandibular* branch of the fifth nerve, which supplies the lower jaw and also gives off the smaller *maxillary branch* to the upper jaw.

The Acustico-facialis Ganglion and Nerves. — As indicated above, the ganglion which gives rise to the VII and VIII nerves is at first in a single mass. During the fourth day, however, the antero-ventral portion separates from the remainder, and gives rise to a nerve which extends chiefly along the hyoid arch, though possessing also a small branch to the mandibular. This is the rudiment of the future VII or *facial nerve.* The remainder of the ganglion gives rise to the VIII or *auditory nerve* which is purely sensory and which communicates with the inner ear, as described below.

The Glossopharyngeal Ganglion and Nerve. — This nerve arises from the IX cranial ganglion, which is situated above the third visceral arch. It appears on the fourth day and extends into this arch. Later another branch enters the second arch, and together they eventually supply the tongue and pharyngeal region.

The Vagus or Pneumogastric Ganglion and Nerve. — On the fourth day an incipient division of the X ganglion becomes clearly marked, separating it into a dorsal portion, the *ganglion jugulare*, and a ventral portion, the *ganglion nodosum.* From the latter there now proceed branches into the fourth and fifth visceral arches, and also a main trunk posteriorly. During the fifth and sixth days this main trunk comes to innervate the heart, the lungs, and the stomach, and at the same time its ganglion (ganglion nodosum) is carried back into the thoracic region. From this region, the proximal portion of the vagus runs

forward to the ganglion jugulare and through it to the medulla. Eventually a small part of this latter ganglion becomes detached as the *ganglion cervicale primum* of the sympathetic system.

THE CRANIAL MOTOR NERVES

The Motor-ocular or III Nerve. — The early development of this cranial motor nerve has already been described. During the fourth day, it passes down beneath the optic stalk, and there enters a ganglion. This receives a connection from the ophthalmic branch of the V nerve, and is known as the *ciliary ganglion*. The III nerve ends by innervating the superior, inferior, and internal rectus, and the inferior oblique muscles of the eye when these develop.

The IV or Trochlearis Nerve. — This motor nerve does not appear until the fifth or sixth day, but will be described at this point. It is peculiar as a motor nerve, in that it arises from the dorsal side of the brain, at the bottom of the isthmus. It has no connection with any ganglion, and ultimately innervates the superior oblique eye muscles.

The VI or Abducent Nerve. — This is a perfectly typical motor nerve, appearing toward the end of the fourth day. It has no ganglion, and arises from the ventral side of the medulla median to the point of origin of the fifth nerve. It innervates the external rectus muscle of the eye.

The XI or Spinal Accessory Nerve. — There is no data on the development of this nerve in the chick (Lillie).

The XII or Hypoglossus Nerve. — This nerve develops during the fourth day from two pairs of ventral roots on the medulla at the level of the third and fourth somites. There are no ganglia, and the roots are evidently serially homologous with the ventral roots of the spinal nerves. The nerve to which they give rise eventually innervates the floor of the pharynx.

THE ORGANS OF SPECIAL SENSE

THE EYE

At the end of the third day the inner wall of the optic cup had thickened, and the whole cup was in the process of enlarging. The lens, meanwhile, had separated from the external ectoderm, and the

side of the lens toward the cup had also begun to thicken. The further development of the eye may be described as follows:

Parts Connected with the Optic Cup. — During the fourth day, pigment begins to appear in the wall of the optic cup nearest the brain; i.e., its outer wall. At the same time, there is developing upon the innermost surface of the inner wall, the *internal limiting membrane*. Beneath this membrane, but still toward the inner side of the inner wall, neuroblast retinal cells in the region of the fundus are beginning to send out axones. These pass over the retinal elements but just beneath the limiting membrane, and find their way out to the optic stalk through the proximal part of the choroid fissure. Here, they proceed among the cells of the ventral wall of the stalk toward the brain, but do not reach it during the fourth day. Later, many more such fibers thus pass through the ventral part of the optic stalk, causing it to swell so that the original internal cavity is obliterated. It may then be termed the *II* or *optic nerve*. In this connection it may further be noted that during the fifth and sixth days the processes of growth occur in such a manner as to alter the relative position of the point of attachment of the optic stalk to the cup. The result is that at the completion of these processes the point in question is no longer at the ventral edge of the cup, but approximately at its center, opposite to the lens.

Subsequent to the fourth day, other changes are also occurring in the walls of the optic cup. As the various cell layers of the retina are formed in the inner wall, this wall shows differentiation into two zones. The central and larger of these, which includes the fundus, is called the *retinal zone;* i.e., the *retina* proper, and it is only within this zone that the above retinal elements are developed. The remainder of the inner wall consists merely of a band around the rim of the cup, and is known as the *lenticular zone*. The line of separation between the two is known as the *ora serrata* (Fig. 193). Within the retinal zone, the outer wall forms the *pigmented layer* of the retina, but never completely fuses with it. In the lenticular zone, on the contrary, fusion between inner and outer walls is complete, pigment penetrates them both, and both remain relatively thin. From this zone, in connection with certain mesenchymal elements, are differentiated the *iris* and the *ciliary processes*. While these parts are forming, the cavity of the optic cup is being filled with a gelatinous matrix containing fibers. Both elements are probably derived from certain

cells of the retinal and lenticular zones, and together are known as the *vitreous humor*. Certain of the fibers of the humor are connected with the ciliary processes, and help to support the lens. Finally the outside of the cup is gradually covered by two
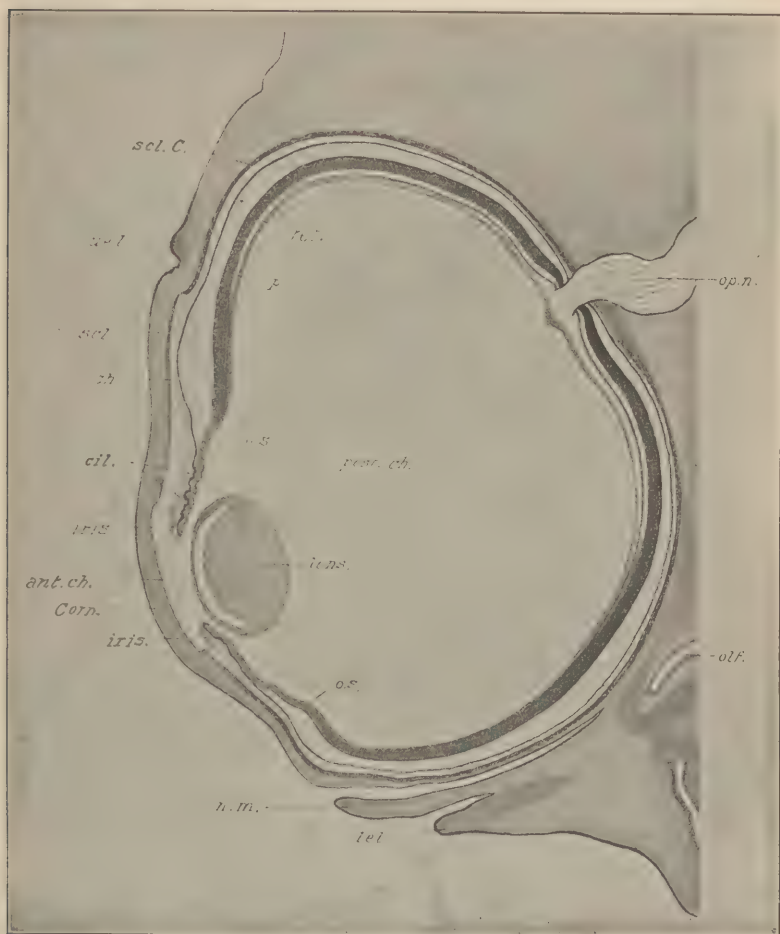
Fig. 193. — Frontal section of the eye of an eight day Chick. From Lillie (Development of the Chick).

ant.ch. Anterior chamber of the eye. *ch.* Choroid coat. *cil.* Ciliary processes. *Corn.* Cornea. *l.e.l.* Lower eyelid. *n.m.* Nictitating membrane. *olf.* Olfactory sac. *op.n.* Optic nerve. *o.s.* Ora serrata. *p.* Pigment layer of the optic cup. *post.ch.* Posterior chamber of the eye. *ret.* Retina. *scl.* Sclerotic coat. *scl.C.* Sclerotic cartilage. *u.e.l.* Upper eyelid.

layers of mesenchymal origin. The inner is the *choroid coat,* and the outer the *sclerotic coat,* the latter being partly cartilaginous.

The Pecten. — This body is also developed in connection with the optic cup and choroid fissure, but is entirely peculiar to the Birds. It has seemed well, therefore, to emphasize it by a separate description. It arises during the fourth day in the form of a blood vessel embedded in mesenchyme. This mesenchymal mass is in the shape of a ridge which enters the cavity of the cup through the choroid fissure near its proximal end. The distal end of the fissure between this mesenchyme and the rim of the cup has, meanwhile, been closed. On subsequent days, the mesenchymal ridge pushes up into the cavity, while at the same time it is being gradually covered over by the in-turning and up-growth of the edges of the choroid fissure on either side of it.
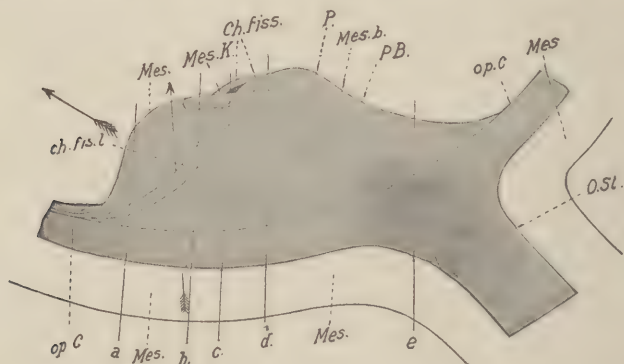
Fig. 194. — Diagrammatic reconstruction of the pecten of the eye of a Chick embryo of 7½ days, incubation. From Lillie (Development of the Chick). After Bernd.

Ch.fis.l. Lip of the choroid fissure. *Ch.fiss.* Choroid fissure. *Mes.* Mesenchyme. *Mes.b.* Upper edge of the mesenchymal ridge covered by the lips of the choroid fissure. *Mes. K.* Thickening of the edge of the mesenchymal ridge. *op.C.* Optic cup. *O.St.* Optic stalk. *P.* Pecten. *P.B.* Base of the pecten.

The arrow indicates the direction of growth of the lips of the choroid fissure over the mesenchymal ridge. The line d shows the plane of the section reproduced in Fig. 195.

This covering soon becomes more prominent than the relatively thin ridge of mesenchyme which it has overgrown, and presently (eighth day) the two parts become indistinguishable. Though remaining constricted at its base, the ridge of fused tissues inside the cavity of the cup continues to grow somewhat, and later becomes folded, assuming the appearance of a fan. It has hence been named the *pecten.* It is very vascular and presumably helps to nourish the retina. The

opening in the choroid fissure between the pecten and the optic stalk furnishes the means of exit for the optic nerve fibers from the retina. A few of these fibers run directly to this point, but the majority come to the base of the pecten, and then run along its sides to the place of exit (Figs. 194 and 195).

The Lens. — At the end of the third day, the inner wall of the lens vesicle had thickened considerably by virtue of the lengthening of its cells. This process continues for several days until the cavity of the vesicle is entirely obliterated. Moreover, inasmuch as the lengthening of the central cells is greater than that of those at the periphery,
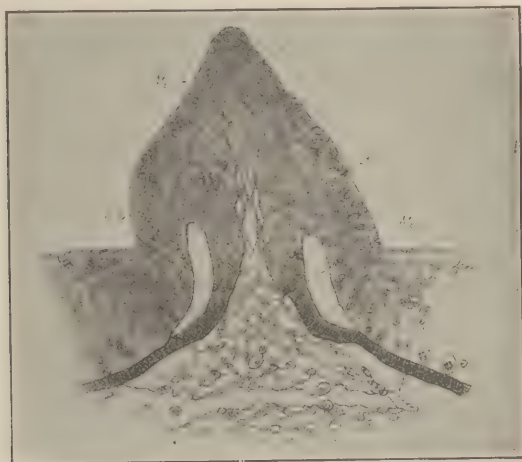
Fig. 195. — Section in the plane of d of Fig. 194, to show the histological structure. From Lillie (Development of the Chick). After Bernd. *Bl.v.* Blood vessel in mesenchymal ridge. *il.* Retinal layer of optic cup. Other abbreviations as in Fig. 194.

the inner surface of the lens becomes distinctly convex (Fig. 193). These lengthened cells of the inner wall form the core of the future lens, while the cells of the outer layer toward the ectoderm form a simple epithelium. The lens now grows, largely by the production of cells at its equator where the inner and outer walls meet. These cells become fiber-like and wrap themselves around the original elements which form the core, thus increasing the size of the lens by the addition of concentric layers of cells.

The Cornea, the Anterior Chamber, and the Lids. — The cornea at first consists merely of the external ectoderm opposite the lens.

On the fourth day, however, this layer is augmented internally by a thin non-cellular layer of mesenchymal origin. On the fifth day, this thickens slightly, and begins to be covered on the side toward the lens by a third layer formed of mesenchymal cells. Later, the middle layer becomes cellular by the migration into it of cells from the mesenchyme, while the third and innermost layer forms a typical epithelium. The latter finally becomes continuous at its edges with the cells of the sclerotic coat. The *cornea* thus constituted arches outward slightly, and thus a chamber is formed between its inner layer and the front of the lens. This is the *anterior chamber,* and it becomes filled with the *aqueous humor.* The *lids* begin to develop about the seventh day as folds of the integument surrounding the cornea (Fig. 193).

The Ear

The Internal Ear. — At the end of the third day, the otocyst, or future internal ear, was in the form of a sac. The uppermost portion of the sac had been slightly constricted away from the lower major portion, and had started to grow upward somewhat as the rudiment of the endolymphatic duct. This upper portion, furthermore, still retained its narrow tubular connection with the exterior (Fig. 183). There is, in these parts, no marked change characteristic of the fourth day. Upon the fifth day, however, the connection of the endolympathic duct with the exterior is entirely lost. Moreover, the opening of the duct into the sac is being gradually shifted ventrally along the median side of the latter. At the same time, the dorsal part of the duct is continuing to grow upward, and expanding to form the *saccus endolymphaticus.* Eventually, this becomes embedded in mesenchyme above the hind brain.

While these events are taking place in connection with the formation of the endolymphatic duct the remaining major portion of the otocyst is developing further, as follows: Upon the early part of the fifth day, there arises from its dorsal half a vertically elongated, hollow out-pushing in the direction of the ectoderm. Then a horizontal out-pushing appears just beneath the first, and therefore at about the equator of the otocyst. Presently a vertical split develops in the ventral part of the vertical out-pushing and soon extends dorsally, thus dividing it into an anterior and a posterior ridge. The anterior, posterior, and horizontal ridges which have thus arisen are the rudiments

of the respective *semicircular canals*. These canals eventually develop by a gradual constricting away of the hollow ridges, so that they become separated from the otocyst everywhere except at their ends. During this process a dilation occurs on each canal to form its *ampulla*. The remainder of the dorsal portion of the otocyst into which the canals open is the *utricle*.

Meanwhile, most of the ventral part of the otocyst has grown downward and also turned backward and toward the median line of the head. Its end forms the *lagena,* and the portion connecting this with the
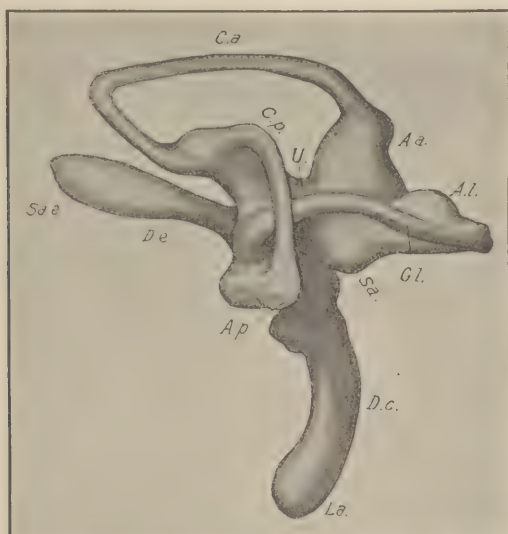
Fig. 196. — Model of the auditory labyrinth of the right side of a Chick embryo of 8 days and 17 hours; external view. From Lillie (Development of the Chick). (After Röthig and Brugsch.).

A.a. Ampulla of the anterior vertical semicircular canal. *A.l.* Ampulla of the lateral horizontal semicircular canal. *A.p.* Ampulla of the posterior vertical semicircular canal. *C.a.* Anterior vertical semicircular canal. *C.l.* Lateral horizontal semicircular canal. *C.p.* Posterior vertical semicircular canal. *D.c.* Ductus cochlearis. *D.e.* Endolymphatic duct. *La.* Lagena. *Sa.e.* Endolymphatic sac. *U.* Utriculus (utricle).

utricle is the *ductus cochlearis*. The *sacculus* arises about the seventh day as a pouch on the median side of the uppermost portion of the ventral part of the otocyst; i.e., just above the point where the latter receives the ductus cochlearis (Fig. 183, *B*).

The parts of the inner ear thus far described constitute the *membranous labyrinth* (Fig. 196). The walls of this labyrinth are com-

posed of epithelium, and its cavity is soon filled with the *endolymphatic fluid*. Except for small areas within the ampullæ and at certain other points, the above epithelium becomes flat. At these points, however, elongated sensory cells end in hairs which project into the fluid, and among these grow the endings of nerve fibers (axones) coming from the VIII cranial ganglion.

On the sixth, day, the mesenchyme which immediately surrounds the developing labyrinth begins to form a membrane (*membrana propria*) in close contact with it. At the same time the more peripheral mesenchyme is forming a cartilaginous case, separated slightly from the labyrinth and its membrane, but following all its contours. The space between the two is called the *perilymphatic space*. It is bridged by tissue which carries the nerves and blood vessels, and is filled by the *perilymphatic fluid* derived from loose mesenchyme tissue left within the space. The cartilaginous case later becomes ossified, and is known as the *bony labyrinth*.

The Middle Ear, or Tubo-Tympanic Cavity. — As was stated in connection with the alimentary tract, the first visceral clefts are closed during the fourth day, and the ventral portion of the pouch of each disappears. The dorsal portion, however, grows up toward the respective otocyst, and during the fifth and sixth days comes between it and the external epithelium. Each pouch then starts to enlarge, and the space within it is the dorso-lateral portion of one of the two *tubo-tympanic cavities*. Meanwhile, beginning on the fourth day, the ventro-median portion of each cavity is developed, as follows. In the antero-dorsal region of the pharynx, a horizontal shelf has grown backward, so as to produce a dorsal chamber virtually separate from the space beneath. Laterally, the part of each tubo-tympanic cavity already developed opens into this newly constituted dorso-median chamber. Then as growth proceeds an increasing portion of this chamber becomes drawn out into the respective cavities. Thus eventually the larger part of each middle ear space is really developed in this manner, rather than directly from the original " gill " pouch. When these processes are complete the median part of the dorso-median chamber still remains as such, while its lateral parts constitute the *Eustachian tubes*. They have a common opening into the mouth by a single median slit-like aperture in the horizontal shelf. As in the case of the Frog, each tubo-tympanic cavity contains a bone, the *columella*. Its development can best be described, however, in connection with the tympanum.

The External Auditory Meatus and the Tympanum. — It will be recalled that the temporary external opening of the first gill pouch occurs only at its dorsal end. Ventrally, however, there is a fusion with the ectoderm which causes the latter to form a vertically elongated pit. When the dorsal perforation closes, that point also is marked by a pit. These pits presently disappear, and on the sixth day the point between them becomes marked by a new depression, the beginning of the *external auditory meatus*. It gradually deepens until, except for a thin layer of mesenchyme, the external ectoderm is in contact with the endoderm of the tympanic cavity. These thin layers of ectoderm, mesenchyme, and endoderm which thus separate the middle ear from the outside, constitute the *tympanum* or ear drum.

To the inside of the tympanum of an adult Bird is attached one end of the columella. The other end is in contact with the wall of the bony labyrinth; i.e., the bony case which finally surrounds the membranous labyrinth. The columella is, therefore, like a bridge stretching across the tympanic cavity from the tympanum to the inner ear. It is chiefly developed from mesenchyme which lies in the dorsal wall of the enlarged tubo-tympanic portion of the gill pouch. This mesenchymal rudiment, it may be noted, is thought to be derived from the dorsal end of the second or hyoid arch. However that may be, as the cavity increases in size, it extends upward on each side of the above mesenchyme until it has surrounded it except at its inner and outer ends. Then as this mesenchyme becomes cartilaginous and finally ossifies, it forms a bone (the columella), occupying the position already described. Lastly it should be added that the inner end of this bone in contact with the labyrinth seems to arise, at least in some Birds, from an element (the *stapes*) which, though at first distinct, eventually fuses with the columella. This stapedial element in the Bird would thus apparently correspond to the opercular element in the ear of the Frog.[1]

The Olfactory Organs. — It will be recalled that, at the close of the third day, the olfactory epithelium consisted of two types of cells: simple epithelial cells and germinal cells. It had also become depressed to form the olfactory pits. During the fourth day this process of depression continues to a considerable extent, and thus the specialized

[1] Some writers recognize a third element, the *stylohyal*, which enters into the formation of the columella of Birds. It must be stated, however, that the exact origins, as well as the homologies of the bones of the middle ear in the various groups of Vertebrates are not yet thoroughly known.

olfactory epithelium lying at the bottom of the pits is carried in some distance from the surface. The epithelium forming the sides of the pits, on the other hand, is unmodified and similar to that outside. The position of the pits has also shifted somewhat with the growing
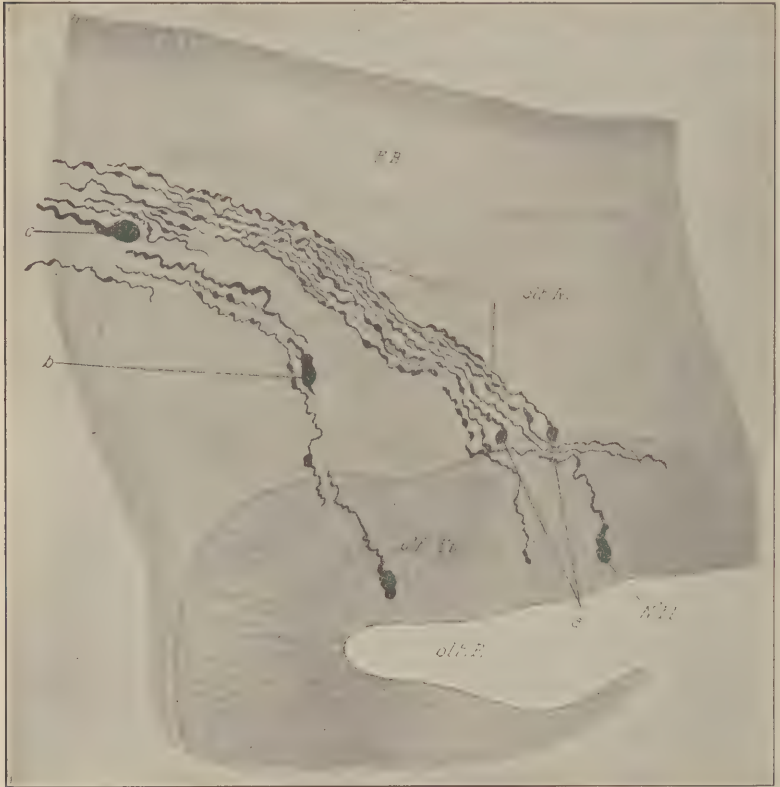
Fig. 197. — Sagittal section through the head of a Chick embryo of 5 days, showing the floor of fore-brain, olfactory pit, and developing olfactory nerve between. From Lillie (Development of the Chick). After Disse.

a. Unipolar neuroblasts near the olfactory epithelium. b. Bipolar cell in the olfactory nerve. c. Unipolar cell near the brain. F.B. Floor of fore-brain. N'bl. Neuroblast in the olfactory epithelium. olf.Ep. Olfactory epithelium. olf.N. Olfactory nerve. olf.P. Cavity of olfactory pit.

of the head, so that their mouths now lie just on the antero-lateral border of the oral cavity.

At the same time that these processes are taking place, the germinal cells referred to are transformed into neuroblasts, and the latter in

turn into typical neurones. On the external side, these neurones send short processes to the surface of the olfactory epithelium. On the other side, they produce axones which extend in toward the brain, the region of whose future olfactory lobes they do not enter, however, until about the sixth day. Along the course of these axones are a few bipolar neurones and also numerous epithelial cells, the latter serving as supporting and sheath cells for the fibers. Both types are said to migrate from the olfactory epithelium, to their final position during the growth of the axones. The axones, together with the other cells just indicated constitute the *I cranial nerve* (Fig. 197).

On the fifth and succeeding days, the nasal cavities continue to deepen somewhat, and become greatly modified in shape. This is partly the result of the appearance of certain folds in the nasal wall; they are the rudiments of the three nasal turbinals, only one of which is finally covered by epithelium of the olfactory type.

While the internal development of the olfactory organ is thus progressing, certain external changes are also going on in connection with the apertures. However, since these changes have more to do with the development of the face than with that of the olfactory organs proper, they will be discussed under the heading of general external changes in Chapter XII.

THE URINOGENITAL SYSTEM

The Excretory System

The Mesonephros. — At the end of the third day the pronephros had virtually disappeared, while the typical mesonephros was beginning to develop, posterior to the twentieth somite. During the fourth day, the primary mesonephric tubules are developed from the most ventral vesicles throughout the greater part of the mesonephric region. The remaining vesicles which occur in every mesonephric segment are, moreover, each giving rise to a tubule. Thus besides the primary tubules, there are formed eventually *secondary* and *tertiary* tubules and sometimes even more, all of a similar nature, developing from the nephrotomal mass opposite each somite. As suggested in the previous chapter, the primary tubules thus formed soon connect directly, through a non-secretory or conducting portion, with the Wolffian duct. The

others as they develop empty into outgrowths from that duct, which receive the name of *collecting tubules* (Fig. 184).

At the time that these tubules are developing, the remaining portion of each vesicle is forming a *Malpighian body* or corpuscle consisting of a glomerulus and its capsule. These Malpighian corpuscles are similar in essential respects to those found in the Frog, and need not be described further.

The Metanephros. — The rudiment of the *ureter* and collecting tubules of the *metanephros*, or permanent kidney, of the Chick appears at the end of the fourth day as a diverticulum from the mesonephric duct. It arises from the dorsal side of this duct just at the point where the latter bends to enter the cloaca. During the fourth day, also, the nephrotomal tissue, just posterior to the thirtieth somite or end of the mesonephros, begins to degenerate for a short distance (Fig. 213, see chapter XIII). Thus anterior to this point, the mesonephros, and any undifferentiated nephrogenous tissue overlying it, become entirely cut off from the nephrotomal tissue posterior to them. The latter tissue thus cut off accompanies the forward growth of the ureter and its collecting tubules, and is destined to form the secreting portion of the entire metanephros (Fig. 213, see chapter XIII).

THE REPRODUCTIVE SYSTEM

The Gonads. — The rudiments of the two gonads appear on the fourth day as thickenings of the peritoneal epithelium on each side of the dorsal mesentery, between it and the respective mesonephros. These thickenings occur just posterior to the origin of the vitelline arteries, and extend for seven or eight somites; i.e., through the posterior half or third of the mesonephric region. Presently primordial germ cells appear in this epithelial tissue, having probably migrated thither as in other Vertebrates. No sex differentiation is apparent at this time.

The Gonoducts. — The future male gonoducts or *vasa deferentia* are the mesonephric ducts whose development has already been described.

The *Oviducts* or *Müllerian ducts* begin their development at this time in both sexes in the form of two ridges, the *tubal ridges*. Each ridge is a strip of thickened peritoneum which appears on the fourth day. It lies on the dorso-lateral face of each mesonephros next to the body wall and near to the Wolffian duct. It is first found at

about the level of the twentieth somite; from this point it differentiates posteriorly (see Fig. 218*A*, Chapter XIII).

THE ADRENALS

These bodies, though not really a part of the renal system, are closely connected with it, and their development may, therefore, best be described at this point.

As in the Frog, the adrenal organs are composed partly of cells derived from the peritoneum, and partly of cells from the sympathetic nervous system. The former element, known as the *cortical substance*, is all that appears during the fourth day. It arises from the body wall near the germinal epithelium, and comes to lie between the Wolffian body and the dorsal aorta.

SUMMARY OF THE CONDITION AT THE END OF THE FOURTH DAY OF INCUBATION

I. THE SOMITES

The number of pairs of *somites* has increased to forty-two, the definitive number of the adult Bird, while the *myotomal, dermatomal,* and *sclerotomal* elements have been developed in each pair. The last named element forms a nearly complete sheath about the nerve cord and notochord, and shows slight indications of the *vertebral segments*. The account of the further development of the myotomal and dermatomal elements is completed in this chapter.

II. THE ALIMENTARY TRACT

The Fore-gut. — The rudiments of the *tongue* have appeared. The *first* and *second visceral clefts* have closed, and the third opened; the *visceral arches* reach their maximum development as such. The *thyroid* has completely separated from the floor of the pharynx. Subsequent development of the tongue and thyroid are indicated in this chapter.

The posterior end of the *laryngotracheal groove* and the *lung rudiments* have separated from the alimentary tract.

The *œsophagus*, the *stomach*, and the *duodenum* have increased in length, and the two latter parts of the tract have developed a curve to

the left. The *liver* has increased in size and come to lie somewhat in the curve of the stomach. The *dorsal pancreatic rudiment* has become a solid outgrowth and a pair of *ventral pancreatic rudiments* have arisen from the *ductus choledochus.* The *spleen* (not really a part of the alimentary tract) has started to develop.

The Mid-gut. — The mid-gut or region of the *small intestine* is now a virtually straight tube open to the yolk only by the relatively constricted aperture of the *yolk-stalk.*

The Hind-gut. — The anterior portion of the hind-gut constitutes the *rectum,* while its terminal portion becomes the *cloaca.* The latter is still separated from the exterior by the *cloacal membrane,* and its posterior part is laterally compressed.

III. THE CIRCULATORY SYSTEM

The Heart. — The *ventricular* region, especially the transverse portion, has expanded and moved posteriorly. The *bulbus arteriosus* has swung toward the median line, and the *auricles* have rotated forward. The *interventricular,* the *interauricular,* and the *cushion septa* are developing.

The Embryonic Arteries. — The *second aortic arches* have disappeared, and the *fifth* and *sixth* pairs have developed. From the latter have arisen the roots of the *pulmonary arteries* which grow out and connect with the rudiments coming from the lungs. The *primary subclavian* and the *sciatic arteries* have appeared, and the latter have given rise to the *umbilical* or *allantoic arteries.* The history of the sciatic and allantoic vessels is concluded in this chapter.

The Embryonic Veins. — The *ring* about the alimentary tract, which is formed in connection with the *vitelline veins,* has been broken by the disappearance of its left half. A fusion of the above vessels has occurred beneath the fore-gut, forming a second ring. The capillaries of the *ductus venosus* among the branches of the liver diverticula are becoming more numerous. Posteriorly, on the ventral side of the mesonephros, the rudiments of the *subcardinals* have become distinct vessels and have acquired direct connections with the posterior cardinals. The *inferior vena cava* has begun to form in the liver and caval fold, and posteriorly has connected with the right subcardinal. The longitudinal vein in the right body wall is disappearing, and the left, having acquired a connection with the allantoic vessels, has become the func-

tional *umbilical vein.* The account of its development is completed. The *pulmonary veins* appear in connection with developing lungs.

The Extra-Embryonic Arteries. — The *vitelline* arteries have fused with one another for a short distance as they leave the aorta. Their branches in the area vasculosa continue to develop in company with the growth of that region, but are without features requiring further note.

The Extra-Embryonic Veins. — The *right anterior vitelline vein* has disappeared, but the *left anterior, posterior,* and *lateral* veins are well developed. Subsequent development of the extra-embryonic veins is included in this chapter.

IV. The Nervous System

The Flexures. — The *cranial* and *cervical flexures* have increased slightly; the *pontine* flexure may be in evidence. The *lateral rotation* of the body is complete.

The Brain. — The *cerebral hemispheres* have increased in size, and their lateral walls are thicker. The *optic lobes* are also becoming steadily more prominent. There are no other marked changes evident at this time.

The Spinal Cord and Spinal Nerves. — There is no special development on the fourth day.

The Cranial Ganglia and Mixed Nerves. — From the *V nerve ganglion* a branch (*ophthalmic*) has extended toward the future beak and another (*mandibular*) toward the angle of the mouth. The *VII nerve ganglion* has become separated from the VIII, and has given rise to the *hyoid* and *mandibular* branches. The *IX ganglion* has sent a nerve into the third arch. The X ganglion has divided into the *ganglion jugulare* and *ganglion nodosum,* and the latter is giving rise to the *vagus nerve.*

The Cranial Motor Nerves. — The *III nerve* has entered the *ciliary ganglion,* and the *VI nerve* have just appeared. The *XII nerve* has also begun to develop.

V. The Organs of Special Sense

The Eye. — Pigment is present in the *outer wall* of the *optic cup.* On the *inner wall* the *internal limiting membrane* is developing and be-

neath this in the region of the fundus, axones of the *retinal neuroblasts* are growing into the optic stalk. The *choroid fissure* has partly closed, and its proximal end is filled with the ingrowing *pecten*. The inner wall of the *lens* is continuing to thicken. The middle layer of the *cornea* has begun to develop.

The Ear. — There is no characteristic change directly connected with the ear at this time. Within the pharynx, however, the formation of the *tubo-tympanic cavities* has begun.

The Olfactory Organs. — The depression of the *pits* has greatly increased, and their openings now lie on the border of the oral cavity. The *olfactory epithelium* is giving rise to the elements of the *I nerve*.

Besides describing the events of the fourth day, this chapter also includes an account of the subsequent development of the nervous system and the organs of special sense.

VI. The Urinogenital System

The Excretory System. — *Primary tubules* have developed throughout most of the *mesonephros*, while *secondary* and *tertiary tubules* are arising. *Collecting tubules* are springing from the Wolffian duct to connect with the two latter types. The *Malpighian bodies* are beginning to appear in the functional portion of the organ. Rudiments of the *metanephros* are evident as a diverticulum from the posterior end of each mesonephric duct. The nephrotomal tissue just behind the mesonephros is beginning to degenerate.

The Genital System. — The *Gonads* are represented by thickenings of the peritoneal epithelium on either side of the dorsal mesentery, and contain *primordial germ cells*. The *oviducts* are present in both sexes in the form of the *tubal ridges*.

VII. The Adrenals

The *cortical substance* of the adrenal bodies appears on the peritoneal wall near the mesonephros.

VIII. The Amnion and Allantois

The *amnion* is completed upon the fourth day, while the *allantois* has pushed out somewhat further into the extra embryonic cœlom.

THE CHICK: DEVELOPMENT DURING THE FIFTH AND SUBSEQUENT DAYS

THE EXTERNAL APPEARANCE

GENERAL

During the fifth day, the cervical flexure reaches its maximum curvature and from then on becomes less and less marked, while the protuberance caused by the mid-brain also attains its greatest relative prominence at this time. The third and last visceral cleft closes during the fifth day, and the future neck is slightly indicated; the first three visceral arches, however, are still somewhat in evidence in this region. The limb buds which were merely rounded swellings on the fourth day are beginning to give evidence of joints.

By the seventh day the second and third arches are no longer visible externally, the heart has moved backward so that the neck is clearly defined, and the external auditory meatus has appeared, as indicated in the previous chapter. The limbs are distinctly jointed, and by the eighth day, the fore limbs begin to appear winglike. Upon the eighth day feather germs are also visible, the tail is relatively much shorter, and the position of the abdominal viscera is quite clearly marked by an external protrusion. From this time on, the embryo gradually assumes a typical birdlike form, one of the most striking changes being the relative increase in the size of the body as compared with that of the head (Fig. 198).

THE FACE

In connection with the development of the nose and mouth, the face undergoes so great a change between the fourth and eighth days, that it seems best to treat the subject separately.

At four days the openings of the olfactory pits are separated by a median projection overhanging the mouth. It is the *naso-frontal*

process. Dorso-laterally each pit is further bounded by the *lateral nasal process* lying between the pit and the antero-dorsal part of the eye. Just below each lateral process there is also another slight outpushing adjacent to the antero-ventral side of the eye, termed the *maxillary process* (Fig. 199). During the fifth day the lateral nasal process of either side becomes more closely united with the maxillary

Fig. 198. — Embryo of 7 days' and 7 hours' incubation x5. From Lillie (Development of the Chick). (After Keibel and Abraham).

process beneath it, the two being separated only by the shallow *lachrymal groove.* At the same time an extension of these united processes crosses each nasal pit and fuses with the frontal process, thus dividing the pits into antero-dorsal and postero-ventral halves. Thereafter as development proceeds the former are carried forward as the *external nares* while the latter are drawn back within the mouth as the *internal nares* (Fig. 200). It is thus evident that the middle portion of the upper jaw is to be derived from the naso-frontal process, and the lateral

parts chiefly from the maxillary process. The lower jaw is molded upon the ventral and main part of the mandibular arches (see below). By virtue of these changes the eighth day finds the nares and rudimentary beak quite clearly defined. Further growth of these parts, accompanied by a relative diminution in the size of the eye and the
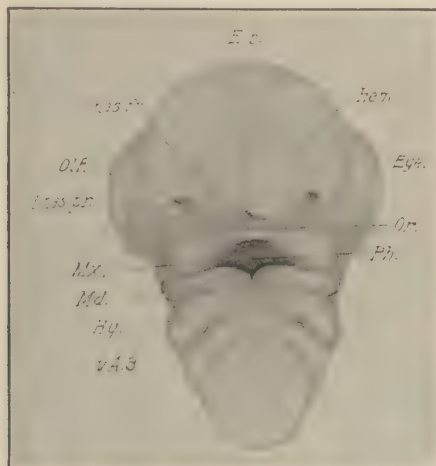
Fig. 199. — Head of an embryo of 4 days' incubation, from the oral surface (N.L. 6 mm.). From Lillie (Development of the Chick).
Ep. Epiphysis. *Hem.* Cerebral hemisphere. *Hy.* Hyoid arch. *l.nas.pr.* Lateral nasal process. *Md.* Mandibular arch. *Mx.* Maxillary process. *nas.fr.* Naso-frontal process. *Olf.* Olfactory pit. *Or.* Oral cavity. *Ph.* Pharynx. *v.A.3.* Third visceral arch.

development of the eyelids, brings the face to the condition found at the time of hatching.

THE SKELETON

It will be possible in a work of this scope to give only an extremely brief account of the development of the skeletal system. For a more extended study, the reader is referred to Lillie's *Development of the Chick,* and the books of reference cited therein.

THE VERTEBRÆ, THE RIBS, AND THE STERNUM

At the end of the fourth day the cephalic portion of each sclerotome was beginning to fuse with the caudal portion of the one anterior to it to form the rudiment of the right or left half of a vertebra. The oc-

currence of these vertebral rudiments thus necessarily alternated with the myotomes. An extension of mesenchyme had also grown up on either side of the nerve cord above both the cephalic and the caudal divisions of every sclerotome, forming in each case the respective posterior and anterior rudiment of a future neural arch. This reversed cephalic and caudal relationship between the original sclerotome on the one hand, and the future vertebræ and their arches on the other,
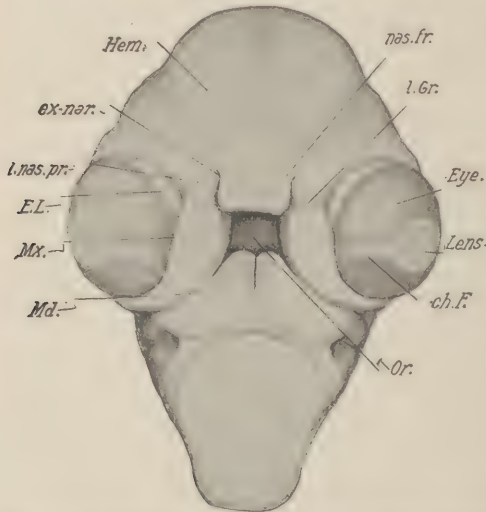
Fig. 200. — Head of an embryo of about 5 days from the oral surface. (N.L. 8 mm.). From Lillie (Development of the Chick).

ch.F. Choroid fissure. *E.L.* Eye-lid (nictitating membrane). *ex.nar.* External nares. *l.Gr.* Lachrymal groove. Other abbreviations as in Fig. 199.

is of course a corollary to the alternative arrangement between the vertebræ and myotomes just indicated.

Upon the fifth day, the fusion of the cephalic portion of each sclerotome with the caudal portion of the next anterior to it is completed. The sclerotomes upon one side of the notochord also have become fused above and beneath it with the corresponding sclerotomes upon the other. Furthermore, as a result of concentration, all of the sclerotomal tissue is beginning to become membranous, and in the region of each future vertebra certain portions of this membrane appear especially condensed. One such condensation surrounds the notochord as a ring, constituting the rudiment of a vertebral *centrum*. Another occurs in each of the up-

growing primordia of the neural arches, and still another arises in the membranous mesenchyme extending outward between the myotomes on either side of the notochord. Each of the latter extensions represents a *costal process*.

During the sixth to the eighth days these costal processes develop further, and in certain regions give rise to the primordia of the true *ribs* (i.e., those separated from the vertebræ by a joint),[1] and also to those of the *sternum*. Thus the membranous primordia of the first pair of such ribs arise from the processes of the cervical (14th) vertebra, while the membrane of the remaining pairs develops from the costal processes of the thoracic vertebræ. The membrane for each half of the sternum is then produced by the fusion of the ventral ends of the

Fig. 201. — The right side of four bisected vertebrae of the trunk of an 8-day Chick. From Lillie (Development of the Chick). After Schauinsland.
caud.v.A. Caudal division of vertebral arch. *ceph.v.A.* Cephalic division of vertebral arch. *N'ch.* Notochord.

fourth, fifth, sixth, and seventh true rib primordia, upon each side, the two halves later uniting. Meantime the *transverse processes* and the *zygopophyses* of the vertebræ are also laid down in membrane, and the membranous ends of the neural arch primordia from either side are united above the nerve cord (Fig. 201). While this is occurring, moreover, cartilage is forming in various regions of the above membranous tissue, which it soon entirely replaces. In this connection it is of interest to note that in the last five pairs of ribs, the dorsal and ventral part of each has its own separate center of chondrification. The sternum itself likewise has two cartilage forming centers, one on

[1] In as much as there appears to be some confusion as to the exact distinction between a true rib and a costal process in the Bird, the writer has chosen to adopt the above parenthetical definition. Upon this basis there are eight pairs of true ribs in the Chick instead of seven, as indicated by those authors who reckon the pair on the 14th cervical vertebra as costal processes.

either side of the median line; these, however, soon fuse. Following chondrification the cartilage is in turn replaced by actual bone; during this procedure the remains of the notochord are completely eliminated. Such ossification is well advanced by the sixteenth day.

Subsequent to this time several of the thoracic and lumbar vertebræ become rather firmly united with one another, and these in turn are fused to the coalesced vertebræ of the sacral region. To this mass there is also added posteriorly a number of the caudal vertebræ, so that a considerable portion of the spinal column is virtually inflexible, a condition peculiar to the Birds. Lastly, the extreme terminal vertebræ are likewise fused into a single piece termed the *pygostyle*.

The Appendicular Skeleton

The Fore-limb. — On the fourth day a concentrated mesenchymal mass probably of sclerotomal origin appears in the base of each fore-limb bud, and on the fifth day there grow out from this membranous mass four processes. One, *the primordium of the limb bones*, grows out into the lengthening wing bud; a second, the *scapula*, grows backward and dorsally above the ribs; a third, the *coracoid*, grows down posteriorly toward the region of the sternum; and a fourth, the *clavicle*, grows in front of the coracoid toward the median line. The last three elements represent the rudiments of the *pectoral girdle*. Centers of chondrification occur in the membranous primordia of the scapula and coracoid on the sixth day, followed later by ossification. The clavicle, on the other hand, ossifies directly from membrane, about the eighth day. Like the coracoid and scapula, the bones of the fore-limb pass through both a membranous and cartilaginous stage previous to ossification. It is interesting to note that in the hand five digits are represented in the membrane, but the first and fifth develop no further.

The Hind-limb. — Like the fore-limb, the parts of the *pelvic* girdle and hind-limb bones arise about the fifth day as four processes from a common mass of mesenchyme in the region of each hind-limb bud. The membranous process representing the limb bones grows out into the bud; another process, the *ilium*, which is elongated in an anterior posterior direction, grows dorsally; a third, the *pubis*, grows antero-ventrally, and a fourth, the *ischium*, grows postero-ventrally. By the eighth day, the distal ends of the pubis and ischium have both

rotated posteriorly so that they are parallel with one another, and with the ilium. Chondrification and ossification follow the membranous stage, and the limb develops in a manner fundamentally similar to that of the fore-limb. There are three *tarsal* elements and five *digits* present in cartilage, but the rudiment of the fifth digit soon disappears. Later the two proximal tarsals fuse with the *tibia,* and the distal one with the three *metatarsals;* subsequent to ossification the latter become united, thus forming with the distal tarsal element the single *tarso-metatarsus.*

The Skull

The Primordial Cranium. — The primordial or cartilaginous cranium of the Chick is first indicated by concentrations of mesenchyme during the fourth and fifth days. Then, during the sixth, seventh, and eighth days, these mesenchymal concentrations develop into the following fused elements of cartilage. Along either side of and encasing the anterior end of the notochord, appear the *parachordal plates.* In the Chick these elements develop from the first as a single piece, and are often known, therefore, as the *basilar plate.* Anterior to it are developed simultaneously upon either side another pair of plates — the *trabeculæ.* Posteriorly, these are continuous with the parachordals, with which they form an angle corresponding to the cranial flexure, while anteriorly, their ends meet and fuse with one another. This fusion then extends somewhat, so that eventually the central space is closed, except for a small opening containing the pituitary body. Thus the trabeculæ and parachordals together form the entire cartilaginous floor of the skull.

At the same time that these plates are forming, cartilage also develops around the auditory sacs and the olfactory organs, forming respectively the *auditory* and *olfactory capsules.* These are in direct continuity eventually with the plates. From the postero-dorsal part of each auditory capsule, processes now grow toward one another and fuse above the hind-brain. Thus is constituted the only portion of the roof of the cranium which is preformed in cartilage. Posterior to each auditory capsule, a dorso-lateral plate of cartilage develops, while anterior to and in contact with the capsule, a transverse partition arises between it and the orbit. This partition extends medially somewhat, so as partially to bound the brain cavity in front. Anterior to the

cranial cavity, midway between the two orbits, and between the nasal capsules, a continuous longitudinal partition appears and fuses ventrally with the trabeculæ. It is the *interorbital* and *internasal septum*.

The remaining part of the skull which is preformed in cartilage is known as the *visceral skeleton* or *cartilaginous splanchnochranium*, and arises from the first three pairs of visceral arches. During the fifth day, these arches are chiefly membranous, and the antero-ventral or distal ends of the first or mandibular pair have fused with one another in the middle line. Subsequent to the fifth day, the ventral or main parts of each mandibular arch become chondrified, and are known as *Meckel's cartilages;* they form the core of each side of the lower jaw. From the proximal (i.e., hinder and upper) end of each of these arches, there develops a tri-radiate piece of cartilage, the *palato-quadrate*, which eventually ossifies as a separate bone. It is termed simply the *quadrate*, and constitutes the articulation between the lower and upper jaws. The second (hyoid) and third visceral pairs of arches later form the hyoid apparatus, consisting respectively of the paired *lesser* and *greater cornuæ* and the two median *copulæ*. Moreover, the upper end of the second arches is thought to give rise to part of the columella, as noted in the account of the ear (Chapter XI).

Altogether, the final bones of the Bird's skull which have been preformed in cartilage are the following: the *basi-occipital*, *exoccipitals*, and *supra-occipitals* about the foramen ovale; the *proötic*, *epiotic*, and *opisthotic* about each auditory capsule; the *basiphenoid*, *orbit-osphenoids*, *alisphenoids*, and *interorbital* and *internasal septum* about the eyes and nasal capsules; the *quadrate*, and *Meckel's cartilages* in connection with the lower jaw; and the *hyoid apparatus* in the region of the throat.

The Membrane Bones. — These are bones which are not preformed in cartilage, but ossify directly from the condensed mesenchyme or membrane. They constitute a good share of the Bird's skull, and begin to develop about the ninth day. The bones thus formed are as follows: the *parietals*, *frontals*, and *squamosals*, forming together the main part of the cranium proper; the *lachrymals*, *nasals*, and *pre-maxillæ*, forming the face and part of the upper jaw; the *maxillæ*, *jugals*, *quadrato-jugals*, *pterygoids*, *palatines*, *parasphenoids*, and *vomer*, forming the rest of the upper jaw and the base of the cranium; and the *angulars*, *supra-angulars*, *operculars*, and *dentals*, forming the covering bones for the lower jaw.

THE ALIMENTARY TRACT

THE FORE-GUT REGION

The development of the mouth proper has already been sufficiently described in connection with the discussions of the alimentary tract and the middle ear in chapter XI, and of the skull in the preceding paragraph. We shall proceed, therefore, to an account of the further development of the remainder of this tract and its appendages.

The Visceral Pouches and Arches.

The Pouches. — At the end of the fourth day, the first and second visceral clefts had closed, and the third had opened; during the fifth
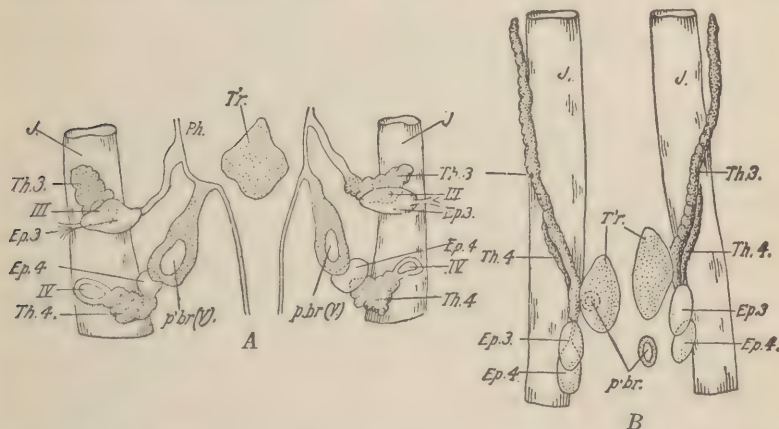
Fig. 202. — Derivatives of the visceral pouches and associated organs, in the Chick. From Lillie (Development of the Chick). After Verdun (Maurer). Combined from frontal sections. A. In embryo of seven days. B. In embryo of eight days.

Ep.3*Ep*.4. Epithelial vestiges derived from ventral portions of the third and fourth visceral pouches. J. Jugular vein. *pbr.*, *p'br.*(*V*). Postbranchial bodies derived from fifth visceral pouch. *Ph.* Pharynx. *Th.*3., *Th.*4. Thymus bodies derived from dorsal portions of the third and fourth visceral pouches. *T'r.* Thyroid body. *III, IV.* Remains of third visceral cleft and position of fourth which never becomes a real cleft.

day, this latter cleft also closes, whereas the fourth pouch, it will be recalled, has never developed an outer opening. About the seventh or eighth day, the third and fourth pouches sever their connections with the pharynx, and thus remain as patches of epithelium in the

mesenchyme of the neck, adjacent to the jugular vein. The dorsal portion of the epithelium from the third pouch then fuses with the dorsal portion from the fourth to form a *thymus* body on each side of the throat of the young Chick. The smaller ventral portions of epithelium give rise to the so-called *third* and *fourth epithelial vestiges*. The fourth pouch also possesses a posterior outpushing sometimes regarded as a vestigial fifth pouch. It separates from the main part of the pouch and forms on each side the *post-branchial* body, (Fig. 202).

The Arches. — The fate of the first three pairs of visceral arches has already been sufficiently described above in connection with the visceral chondrocranium. The fourth pair of arches never develop beyond a mesenchymal state and eventually disappear. The fifth pair are vestigial and even more transitory.

The Respiratory Tract. — At the end of the fourth day, the respiratory tract consisted of the glottis, the larynx, the trachea, and a pair of posterior outgrowths from the latter designated as the rudiments of the lungs. All these parts, having arisen from the foregut, are necessarily lined by endoderm. Upon the fifth day, however, the mesenchyme about them begins to condense to form true mesoderm, through which the lung rudiments continue to grow posteriorly as a pair of tubes. Upon the sixth day, these tubes begin to branch, and thus it appears that the original rudiments really represented the lining of only the two main *bronchi*. Their branches then constitute the lining of the various minor bronchial tubes which finally terminate in the minute air cells or *alveoli*. The above mesoderm in the region of the larynx and the trachea gives rise eventually to the cartilages and muscles of these organs. Further back, it surrounds the endodermal lining of the bronchial tubes and alveoli, and ultimately forms the connective tissue substance of the lung. Through this tissue the blood vessels later ramify among the air cavities.

In the case of the Bird, besides these typical respiratory chambers, there are also connected with the lungs the various *air sacs*. These arise as outgrowths from the two original bronchi and from their primary branches. The rudiments of the abdominal and cervical sacs are said by some to be distinguishable as early as the fifth day, while the others appear somewhat later (Fig. 203). In the course of development these peculiar sacs which have thus originated, gradually push their way to their respective positions among the viscera. Here they

come to occupy considerable space, while a branch of the interclavicular sac extends eventually even into the upper bone (humerus) of each wing. The function of these organs is obviously to give lightness to the Bird's body.

Finally, in connection with the development of the respiratory system, it is to be noted that about the fifth day, the glottis begins to close. Both larynx and glottis later become entirely shut, but subsequent to the eleventh day, the opening is gradually reëstablished.
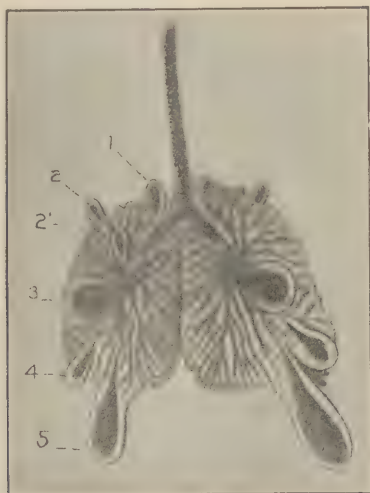
Fig. 203. — Lungs and air-sacs of a Chick embryo of about 10 days. From Lillie (Development of the Chick). After Selenka.
1. Cervical air-sac. 2 and 2¹. Interclavicular air-sac. 3. Anterior thoracic air-sac. 4. Posterior thoracic air-sac. 5. Abdominal air-sac.

The Œsophagus, the Stomach, and the Duodenum. — At the end of the fourth day, the œsophagus was a straight tube, while the region of the stomach and duodenum was indicated by a slight curvature to the left. The œsophagus does not alter much on the fifth day, except to continue to elongate. The stomach, however, is becoming distinguished from the duodenum by its greater dilation. Also, at the extreme left of the gastric duodenal curve, a slight pouch is forming. This marks the end of the gastric region. Later, this pouch enlarges to form the muscular *gizzard*, while the part between it and the œsophagus develops the gastric glands and comprises the *proventriculus*. The

crop is evident by the eighth day as a dilation of the œsophagus at the base of the neck. Anterior to the crop at that time, the lumen of the œsophagus is temporarily closed.

The *duodenum* is not very clearly defined on the fifth day, but shortly afterward it begins to develop as a loop in the tract just beyond the gizzard. From the gizzard, the proximal limb of the loop descends a short distance, and then bends upward to form the ascending branch. Ultimately the pancreas comes to lie in between the limbs of this loop. The end of the ascending branch marks the termination of the original fore-gut region and the beginning of the small intestine (Fig. 204).
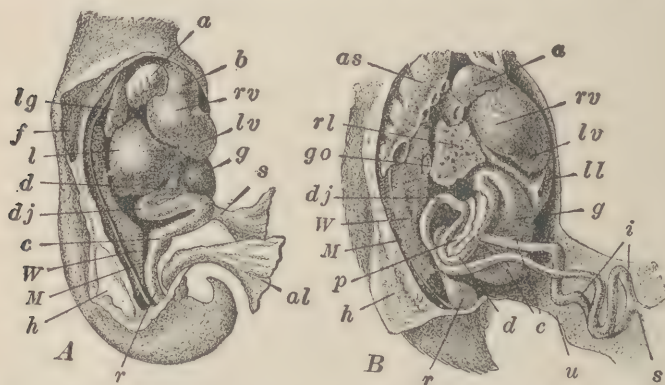
Fig. 204. — Partially dissected viscera of the Chick, from the right side. From Kellicott (Chordate Development). After Duval. A. Of a six-day Chick, enlarged slightly less than six times. B. Of a thirteen-day Chick, enlarged two and one half times, showing the elongated intestine and its extension into the umbilical stalk.

a. Right auricle. *al*. Allantois. *as*. Abdominal air-sac. *b*. Bulbus arteriosus. *c*. Caecal processes. *d*. Loop of duodenum. *dj*. Duodenal-jejunal flexure (a relatively fixed point during the elongation of the intestine). *f*. Fore-limb bud (cut through). *g*. Gizzard. *go*. Gonad. *h*. Hind-limb bud (cut through). *i*. Loops of small intestine. *l*. Liver. *lg*. Lung. *ll*. Left lobe of liver. *lv*. Left ventricle. *M*. Rudiment of Mullerian duct (tubal ridge). *p*. Pancreas. *r*. Rectum. *rl*. Right lobe of liver. *rv*. Right ventricle. *s*. Yolk-stalk. *U*. Umbilical stalk. *W*. Wolffian body or mesonephros.

The Liver. — On the fifth and subsequent days, as on the fourth day, development of the liver consists chiefly in further growth in size. This is accomplished as already indicated by continuous branching and anastomosing of the original diverticula together with the accompanying blood capillaries. These diverticular branches are at first solid, but on the fifth day many of them have acquired a lumen, and this process continues as growth proceeds.

As regards the bile ducts, it is to be noted that on the sixth day

the common duct disappears, and the two bile ducts which emptied into it again empty directly into the duodenum.

The Pancreas. — The pancreas at four days, it will be recalled, consisted of three separate outgrowths: a dorsal one from the wall of the duodenum opposite the common bile duct, and the beginnings of two ventral ones from the duct itself. During the fifth day all three diverticula continue to grow and branch (Fig. 205). On the sixth
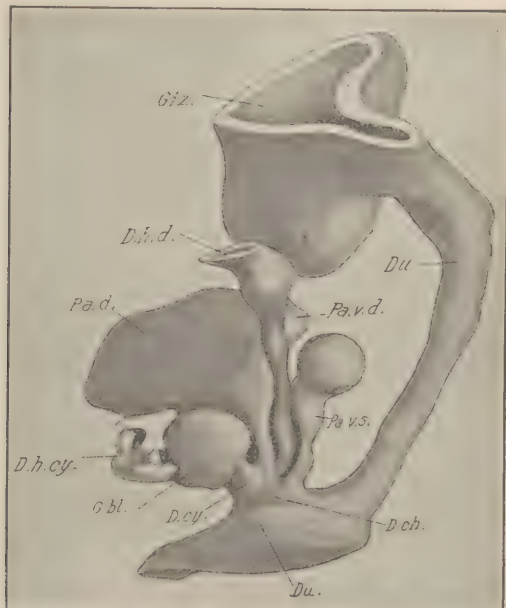
Fig. 205. — Reconstruction of gizzard, duodenum, and hepato-pancreatic ducts of a Chick embryo of 124 hours. From Lillie (Development of the Chick). After Brouha.

D.ch. Ductus choledochus. D.cy. Ductus cysticus. D.h.cy. Ductus hepato-cysticus. D.h.d. Dorsal or hepato-enteric duct. Du. Duodenum. G.bl. Gall bladder. Giz. Gizzard. Pa.d. Dorsal pancreas. Pa.v.d. Right ventral pancreas. Pa.v.s. Left ventral pancreas.

day, the right ventral pancreatic mass becomes united with the dorsal, whose duct shifts ventrally on to the left side of the duodenum. As noted above, the common bile duct disappears at this time, and thus the two ventral pancreatic ducts come to open directly into the intestine. Later, the left pancreas becomes fused with the other two, and there remains a single glandular mass lying in the loop of the duodenum. Its three ducts continue to remain separate, however, and they open into the distal limb of the duodenal loop near the bile ducts.

THE MID-GUT REGION

It has been indicated that the mid-gut or rudimentary small intestine begins at the end of the duodenum. At the close of the fourth day, it was noted that it extended from this point as a virtually straight tube across the region of the umbilicus to the beginning of the tail fold and hind-gut. In about the middle, it gave off the yolk-stalk. During the fifth day a very slight downward bend (the *duodeno-jejunal flexure*) appears just at the point where the duodenum ends and the mid-gut begins. From this bend, the latter extends postero-ventrally for about half its length; at this point, as noted, it connects with the yolk-stalk. It then ascends again to its termination, which is now marked by a small bilateral swelling, the rudiment of the *intestinal cæca*. The entire mid-gut region thus indicated is still quite short, and its dip down into the umbilical stalk very slight.

On the sixth day, however, the ventral dip of the small intestine reaches well down into the above stalk, thus forming in the intestine as a whole a second distinct loop (Fig. 204, *A*). The latter soon becomes much more pronounced than the duodenal loop, and during later development acquires numerous convolutions (Fig. 204, *B*). These convolutions lie within the umbilical stalk until about the eighteenth day and are then drawn into the body. They are soon followed by the remains of the yolk-sac. The intestinal cæca which were barely indicated on the fifth day ultimately grow out into two fingerlike processes.

THE HIND-GUT REGION

On the fifth day, as on the fourth, there is no particular change in the rectum. On the seventh and eighth days, however, its cavity becomes occluded. Later, the lumen is restored except for a small plug separating it from the cloaca, and just anterior to this plug a slight dilation develops. This dilation is the *coprodæum*. The plug persists until about the time of hatching.

The chief change in the cloaca during the fifth day is the fusion of the laterally compressed walls of the posterior part. During subsequent development, a cavity is reëstablished in the postero-dorsal part of this closed portion; it constitutes the *bursa Fabricii* of the adult. This is a sac which remains separate from the original cloaca, but which opens into another cavity, communicating directly with the exterior.

This latter cavity is the *proctodæum*, and has arisen by an outpushing of the ectodermal walls around the edges of the anal plate or cloacal membrane (Fig. 206. Compare Fig. 171, chapter X). At hatching the latter disappears and thus the proctodæum is finally placed in communication with the original embryonic cloaca minus the posterior portion of the latter which went to form the bursa Fabricii. At about
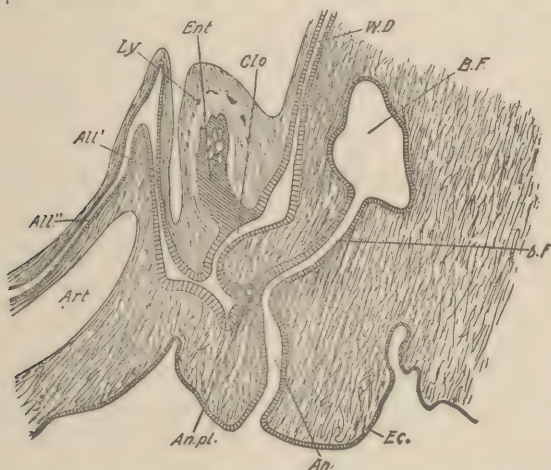
Fig. 206. — Chick embryo of 11 days, sagittal section through the region of the cloaca. Reconstructed from several sections. (After Minot). From Lillie (Development of the Chick). Anterior end toward the reader's left.

All'. Ascending limb of the allantois. *All''*. Descending limb of the allantois. *An*. Anal invagination, or proctodæum. *An.pl*. Anal plate or cloacal membrane. *Art*. Umbilical artery. *B.F*. Bursa Fabricii. *b.f*. Duct of the bursa. *Clo*. Cloaca; i.e., the urodæal portion. *Ec*. Ectoderm. *Ent*. Entoderm of the rectum. *Ly*. Nodules of crowded cells, probably primordia of lymphoid structures in the wall of the large intestine. *W.D*. Wolffian duct.

the same time the plug which closes the rectum disappears. Thus, the adult cloaca consists of three parts, the coprodæum, a part of the original cloacal chamber now called the *urodæum*, and the proctodæum. The latter opens to the outside through the *anus*.

THE CIRCULATORY SYSTEM

The Heart

During the fourth day a series of changes in the position of the various parts of the heart in relation to each other were indicated. During the fifth day these changes progress rapidly, and upon the sixth day are virtually completed.

Besides these movements, there were also noticed on the fourth day the beginnings of certain partitions within the heart. These were the interauricular, the interventricular, and the cushion septa. During the fifth and part of the sixth days, all these are practically completed. This process involves, first, the meeting of the two parts of the cushion septum so as entirely to divide the auricular canal into right and left channels. The auricular septum then unites with the cushion septum on the dorsal side of the latter, while the ventricular septum joins it from below. These fusions, though described separately, occur more or less simultaneously (Fig. 186, *F*).

It remains to be noted in this connection that the auricular septum never becomes entirely complete during embryonic life; instead, it remains fenestrated so that the right and left auricles are only partially separated. These perforations correspond to the *fenestra ovale* in the heart of the Mammal and their physiological significance is described below. There is also a small ventricular foramen whose final closure will be described presently in connection with the development of the aortic division of the bulbus.

This completes the description of the septa within the heart proper. Upon the fifth day, however, another septum develops within the truncus arteriosus. It appears first at the anterior end of this vessel in such a position as to separate the orifice leading to the sixth aortic arches and hence to the pulmonary arteries, from that which leads to the third and fourth aortic arches. This partition then grows backward through the distal portion of the bulbus, and on the sixth and seventh days it connects with a septum which has formed within the proximal portion of that vessel. Thus a continuous somewhat spirally twisted partition has been produced extending through the truncus and bulbus clear in to the interventricular septum of the heart. It is to be noted that the entire bulbus, though now ventral, still lies somewhat to the right of this latter septum. Nevertheless, the fusion of the bulbus septum and interventricular septum is effected in such a way that in connection with subsequent changes in the cushion septum the aortic division (i.e., the division from the third and fourth arches) of the bulbus comes to open through the foramen in the ventricular septum directly into the left ventricle. The pulmonary division, on the other hand, continues to open into the right ventricle (Fig. 207).

Subsequent to the fifth day also, certain other changes are completed as follows. The *semilunar valves* develop in both the aortic and pul-

monary divisions of the bulbus, and the parts of that vessel proximal to these valves are incorporated into the ventricles. The two divisions of the bulbus and truncus arteriosus distal to this point are gradually
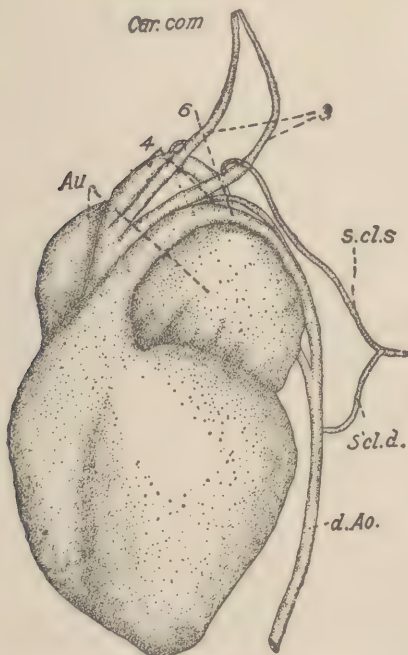
Fig. 207. — The heart and aortic arches of a Chick embryo the latter part of the sixth day. From a dissection. From Lillie (Development of the Chick). After Sabin.
Au. Auricles. *Car.com.* Common carotid artery. *S'cl.d.*, *S.cl.s.* Primary and secondary subclavian arteries. 3, 4, 6. Third (carotid), fourth (systemic), and sixth (pulmonary) aortic arches.

separated so as to form distinct vessels; i.e., the proximal portions of the *aortic* and *pulmonary arteries*. Finally, as noted in a previous chapter, the sinus venosus becomes a part of the right auricle, into which empty all the systemic veins.

THE EMBRYONIC BLOOD VESSELS

The Arteries.

The Aortic Arches. — At the end of the fourth day, the pairs of aortic arches which remained fully developed were the third, fourth,

and sixth. The third pair, it will be recalled, ran upward from the ventral aorta, and continued anteriorly as the internal carotids, while posteriorly the dorsal end of each of these arches was still connected with the dorsal end of each fourth arch. From the base of each of the third arches, on the other hand, another vessel ran forward as an external carotid.

Upon the fifth day two further changes are initiated as follows. First, on each side, the portion of each dorsal aorta between the third and fourth arches begins to disappear. Secondly, the fourth arch on the left side diminishes in size (Fig. 208). By the eighth day the changes thus begun have been completed, and hence it happens that on the left side, the entire fourth arch together with the dorsal aorta

Fig. 208. — Reconstruction of the aortic arches of a 6-day Chick embryo from a series of sagittal sections. From Lillie (Development of the Chick).
A. Left side. B. Right side. Car.com. Common carotid. Car.ext. External carotid. Car.Int. Internal carotid. D.a. Ductus arteriosus (Botalli). 3, 4, and 6. Third, fourth, and sixth aortic arches.

between it and the sixth arch has vanished. On the right side the dorsal connection between the third and fourth arches is gone, but the fourth arch itself is well developed. It persists as the *main systemic arch* of the adult (Fig. 187, B). It is to be noted that the Bird differs from the Mammal in that in the latter, it is the left arch which remains. All parts of the sixth arches continue to be well developed on both sides throughout embryonic life. At the time of hatching, however, the upper portion of each vessel between the origin of the pulmonary arteries and the dorsal aorta (i.e., the ductus Botalli, indicated above) becomes atrophied and remains only as an occasional vestige in the adult.[2]

Finally, it must be remembered that while these changes are occurring, the head of the Bird is being separated from the body by the

[2] In the Mammal, a remnant of the left ductus Botalli always persists.

development of the neck. This process results in the backward movement of the heart and all its arches, so that by the time they have reached the stage indicated on the eighth day, they lie entirely within the thorax. The carotids, on the other hand, are elongated into vessels which pass forward into the head. In this connection, and as a further result of the changes cited above, another carotid arises meanwhile upon each side, as follows. It is evident that as soon as the dorsal connection between the third and fourth arches is gone, all of the blood for both external and internal carotids will pass through the base of the third arch. This base then tends to lengthen somewhat, concurrently with the general elongation, and thus forms a relatively short new vessel, the *common carotid* (Figs. 208, *A* and 209).

The Physiological Significance of the Embryological Structure of the Heart and Aortic Arches. — Before considering the remainder of the blood vessels, it seems well to digress at this time in order to point out the physiological significance of the heart and its arches as they have just been described.

The heart, as has been seen, becomes virtually four chambered. It fails to become entirely so during embryonic life, however, because of the persistence of the fenestra in the interauricular septum. This fact, as well as the existence of the dorsal portions

Fig. 209. — Diagram of the aortic arches of Birds and their fate. From Lillie (Development of the Chick). After Boas.

Car.com. Common carotid. *Car.ext.* External carotid. *Car.int.* Internal carotid. *D.a.* Ductus arteriosus. *L.* Left. *p.A.* Pulmonary artery. *R.* Right. 1, 2, 3, 4, 5, and 6. First, second, third, fourth, fifth, and sixth aortic arches.

of the sixth arches, i.e., the ductûs Botalli, is correlated with the embryonic method of aerating the blood. This becomes clear upon a consideration of what this method involves, as follows:

It is obvious that previous to hatching the lungs of the Bird cannot function. Instead, as has already been noted, respiration is effected chiefly through the allantois. There now remains to be described the relationship which the interauricular fenestra and the ductûs Botalli bear to the distribution of the different classes of blood. The fully

aerated blood from the allantois, the nutrient laden blood from the
yolk-sac, and a relatively small amount of strictly venous blood from
the posterior part of the body become mixed in the ductus venosus,
and from thence are poured together into the right auricle. The struc-
ture of this auricle is then such that apparently most of this blood is
turned away from the right ventricle and guided through the interauric-
ular fenestra into the left auricle. From here it passes into the left
ventricle, and thence through the aortic division of the bulbus and
truncus arteriosus into the third and right fourth aortic arches. The
third arches, as has been seen, convey this blood, for the most part
newly oxygenated and full of nutriment, straight to the head; the
rest passes through the right fourth arch and backward along the dorsal
aorta. On its way, however, it becomes mixed with blood which has re-
turned from the head entirely without oxygen or nutriment and loaded
with carbon dioxide; this occurs as follows: As the unaerated blood
from the head passes into the right auricle it in turn is diverted, pre-
sumably by the direction of its entrance as well as by the structure of
the cavity. Its course, in contrast to that of the blood from the pos-
terior regions, however, carries it away from the interauricular fenestra
and into the right ventricle. From here it passes out through the
pulmonary division of the bulbus and truncus arteriosus, and thence a
slight part of it flows through the small pulmonary arteries into the
rudimentary lungs. The larger part, however, continues through the
dorsal portions of the sixth arches (i.e., the portions constituting
the ductûs Botalli) into the dorsal aorta; here, as indicated above,
it inevitably mixes with the aerated blood from the right fourth arch.
Some of this mixture then supplies the body posterior to the head.
The larger share of it, however, eventually reaches again the walls
of either the allantois or the yolk-sac, where it receives respectively
oxygen or food material, and is returned to the heart in the manner
already noted. From this description it is evident that the blood
received by the posterior part of the body during embryonic life is not
so rich in oxygen or nutriment as that which flows to the head and
fore-limbs. This fact very probably accounts for the more rapid
development of the latter region.

It is also clear from this account that all that is necessary to achieve
the adult circulation at hatching is the completion of the interauricular
septum and the atrophy of the ductûs Botalli. It may be noted in
passing that a similar pair of adjustments is necessary in Mammals at

birth. In Man one or both of these changes sometimes fails to take place, resulting presumably in a mixture of oxygenated and non-oxygenated blood throughout the body. This, in connection with certain other defects, is said to be sometimes responsible, at least in part, for so-called congenital *cyanosis*. Infants so afflicted are known as " blue babies," and seldom live very long.

The Subclavian Arteries. — The primary subclavian arteries arise as outgrowths from the eighteenth segmental arteries. On the fifth day, however, each primary subclavian receives a secondary branch from the base of the respective common carotid (Fig. 207). These new branches then develop, while the original connections with the dorsal aorta through the segmental arteries become atrophied. Thus the *permanent subclavians* eventually arise from the carotids in the Bird. These arteries, of course, supply the wings, and in so doing, develop various branches. It will not be advisable, however, to follow them further in detail.

The Remaining Arteries. — The only other major arteries whose development has not already been indicated in the account of the fourth day, are those which supply the intestine. These are the *anterior mesenteric*, the *cœliac* and the *posterior mesenteric*. The anterior mesenteric arises from the single stem of the vitelline arteries just beyond the point where this leaves the dorsal aorta. The other two develop directly from the latter vessel. These three arteries appear during the fifth and subsequent days (Fig. 210).

The Veins.

The Vitelline Veins. — At the end of the fourth day, a second venous ring had been formed about the intestine by a fusion of the vitelline veins for a short distance beneath it. This second ring was beginning to be destroyed by the disappearance of its right side, and during the fifth day, this side is completely obliterated. From a review of the previous development of this region, it will be evident that the condition of the vitelline veins at this point has now become as follows. The two veins unite just in front of the anterior intestinal portal, and ventral to the intestine, to form a single trunk, which is really a posterior continuation of the ductus venosus. This trunk runs forward beneath the intestine for a short distance, and then curves upward and to the left. It next turns sharply to the right and crosses over the intestine dorsally; finally it bends immediately forward again

and runs into the liver (Fig. 188, E). During subsequent stages as the anterior intestinal portal continues to move backward, it is closely followed by the fusion of the vitelline vessels. Indeed before very long this fusion passes beyond the region of the intestinal portal, and thus the single ductus venosus, or vitelline trunk, comes to extend a considerable distance into the umbilicus before dividing into its two branches.

The Hepatic Portal System. — It will be recalled that within the liver the ductus venosus receives numerous capillaries. These capillaries
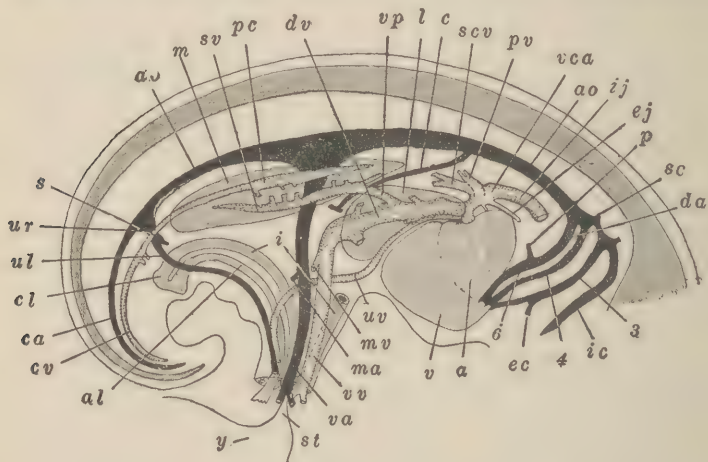
Fig. 210. — Diagrammatic lateral view of the chief embryonic blood vessels of the Chick, during the sixth day. From Kellicott (Chordate Development). After Lillie.

a. Auricle. *al.* Allantoic stalk. *ao.* Dorsal aorta. *c.* Cœliac artery. *ca.* Caudal artery. *cl.* Cloaca. *cv.* Caudal vein. *da.* Ductus arteriosus. *dv.* Ductus venosus. *ec.* External carotid artery. *ej.* External jugular vein. *i.* Intestine. *ic.* Internal carotid artery. *ij.* Internal jugular vein. *l.* Liver. *m.* Mesonephros. *ma.* Mesenteric artery. *mv.* Mesenteric vein. *p.* Pulmonary artery. *pc.* Posterior cardinal vein. *pv.* Pulmonary vein. *s.* Sciatic artery. *sc.* Subclavian artery. *scv.* Subclavian vein. *st.* Yolk-stalk. *sv.* Subcardinal vein. *ul.* Left umbilical artery. *ur.* Right umbilical artery. *uv.* Left umbilical vein. *v.* Ventricle. *va.* Vitelline artery. *vca.* Anterior vena cava (anterior cardinal vein). *vp.* Posterior vena cava. *vv.* Vitelline vein. *y.* Yolk-sac. 3, 4, 6. Third, fourth and sixth aortic arches.

increase during the fifth day, while at the same time the main channel of the vein within the liver begins to disappear. This is brought about through the gradual occlusion of this channel by means of strands of the hepatic substance which grow into and across it. On the fifth day also, a vessel starts to develop in the dorsal mesentery of the gut; it is the *mesenteric vein*, and presently acquires a connection with the

vitelline trunk at about the region of the pancreas. By the seventh day the occlusion of the main part of the ductus venosus within the hepatic substance has been completed. From now on, therefore, the blood enters the liver by the remaining posterior half of this vein, is distributed through the hepatic capillaries, and is finally collected again to enter the now separate anterior half of the same vessel through two main branches. When development has reached this stage the posterior half of the ductus venosus may be termed the *hepatic portal vein*, which receives the mesenteric vessel as its chief tributary. The two branches entering the anterior half of the ductus venosus, upon the other hand, constitute the *hepatic veins* (Fig. 188, *F*).

Upon the fifth and immediately subsequent days the blood which enters the liver circulation is largely from the yolk-sac. Before long, however, the mesenteric vein has begun to send out branches which develop simultaneously with the various digestive organs and spleen. Thus these organs send an ever-increasing supply of blood through the hepatic portal vein to the liver. When the yolk-sac finally disappears they become the sole source of the blood which passes through the hepatic capillaries. The complete system of circulation which is developed in this manner is then called the *hepatic portal system*.

The Fate of the Cardinals and Development of the Caval and Renal Veins. — On the fourth day, the subcardinals lying ventral to the mesonephros have direct connections with the posterior cardinals lying dorso-lateral to it. Upon the fifth day, however, these connections are severed and new ones established through capillaries within the mesonephric substance. At the same time, the subcardinals fuse with one another near their anterior ends, and the connection of the right one with the posterior end of the vena cava inferior (established on the fourth day) becomes larger (Fig. 211). Thus a part of the blood in the posterior cardinals now passes through the mesonephros and by way of the subcardinals and vena cava inferior to the heart. In other words, there is in the embryo of the Bird a typical *renal portal* circulation. On the fifth day also, or late upon the fourth, the *subclavian veins* begin to develop in connection with the fore-limb buds. They arise as branches of the posterior cardinal veins, a short distance behind the junction of the latter with the Cuvierian ducts.

Upon the sixth day, the section of each posterior cardinal between the entrance of the respective subclavian vein and the anterior end of the mesonephros disappears, thus forcing all the blood from the pos-

terior part of the body to traverse the renal portal channels. In this manner also that portion of each posterior cardinal anterior to the entrance of the subclavian becomes simply the proximal part of the latter vessel. From this time on, the ducts of Cuvier which now receive the jugulars (anterior cardinals) and subclavians, may be
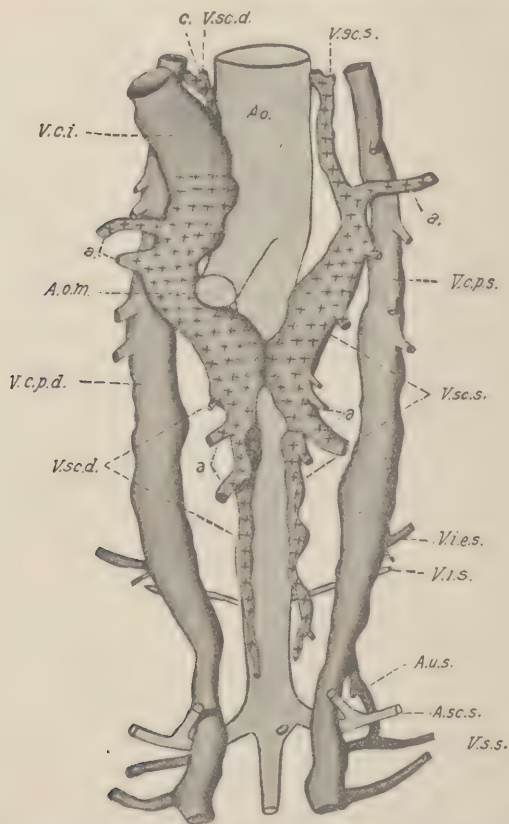
Fig. 211. — Reconstruction of the venous system of a Chick of 5 days. Ventral view. From Lillie (Development of the Chick). After Miller.

a. Mesonephric veins. *Ao.* Aorta. *A.o.m.* Omphalomesenteric artery. *A.u.s.* Left umbilical artery. *A.sc.s.* Left sciatic artery. *V.c.p.d.,s.* Right and left posterior cardinal veins. *v.c.i.* Vena cava inferior. *V.sc.d.,s.* Right and left subcardinal veins.

termed the *anterior* or *superior caval veins*. At about this stage also, the anterior portion of the ductus venosus, which receives the two hepatic veins and the posterior vena cava (vena cava inferior), may be

said to have become merely the anterior end of the latter vessel. Thus the posterior caval vein, like the two anterior cavals, now opens directly into the right auricle.

While the above changes are occurring subsequent to the fifth day, there are a pair of new veins arising in connection with the metanephros or permanent kidney. These are the *renal veins* which presently take blood from the permanent kidney to the anterior fused portion of the
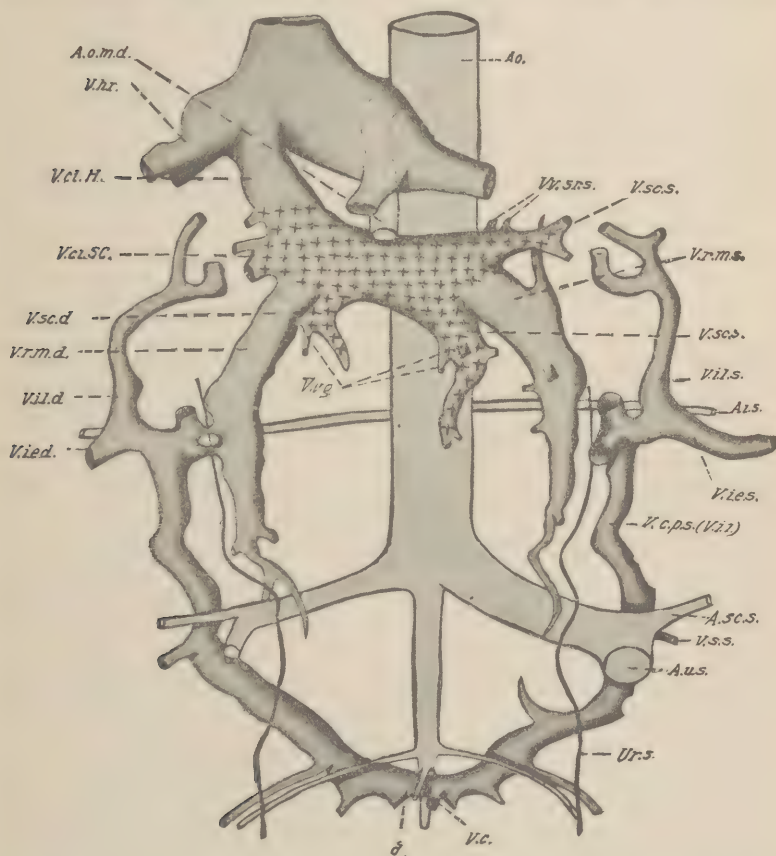
Fig. 212. — Reconstruction of the venous system of a sparrow embryo, corresponding to a Chick of about 14 days. From Lillie (Development of the Chick). After Miller.

V.c.i.H. Intra-hepatic part of the vena cava inferior. *V.c.i.SC.* Part of the vena cava inferior derived from the subcardinal vein. *V.v.g.* Genital veins. *V.i.e.d.,s.* Right and left vena iliaca externa. *V.i.i.* Vena iliaca interna, (or *V.c.p.s.* Posterior part of the cardinal). *V.i.l.d.,s.* Right and left vena intervertebralis lumbalis. *V.r.m.d.,s.* Right and left great renal veins.

subcardinals (now really the posterior part of the posterior vena cava).
Just anterior to the kidney these renal veins also later establish direct
connections with the posterior cardinals. Thus a new channel is
formed for the blood from the posterior part of the body via the
cardinals and the anterior portion of the new renal veins to the
posterior vena cava (Fig. 212). At the same time that this is occur-
ring, the mesonephros together with the renal portal system is disap-
pearing. While the latter exists, however, it is essentially similar to
the permanent system of the same name in the Frog and other more
primitive Vertebrates, thus affording an excellent example of recapitu-
lation. It remains to note that the hinder portions of the posterior
cardinal veins persist in the adult Bird as the *iliac veins*, receiving
branches from the hind-limbs. Also in subsequent stages, branches
from the cardinals fuse with one another medially at the posterior end
of the body and give rise to the *caudal vein*.

THE BODY CAVITIES

From previous discussion, it will be recalled that the space surround-
ing the heart has been designated as the pericardial cavity. Up to this
time, however, there has been no mention made of any separation of
this cavity from the *peritoneal* or general body cavity behind it. It
now remains to describe how this separation is effected, together with
the simultaneous closing off of a third space, the pleural cavity (see
below). It will then be possible in conclusion to show also how the
walls of the pericardial cavity come to form the independent peri-
cardial sac of the adult bird.

The Separation of the Pericardial, Peritoneal and Pleural Cavities

The separation of the peritoneal and pericardial cavities is chiefly
brought about by the development of a partition known as the *septum
transversum*. This so-called septum in turn is composed of three parts,
two of which have already been mentioned. The entire septum then
is made up as follows: First, there is a median mass consisting of the
liver and the sinus and ductus venosus, together with the dorsal and
ventral ligaments which unite the liver to the gut and to the ventral
body wall. Second, there are the lateral mesocardia lying obliquely

in an anterior posterior direction and extending laterally from this mass to the body wall. Above and below the lateral mesocardia, the pericardial cavity still communicates posteriorly with the peritoneal or general body cavity. About the fifth day, however, the ventral communication begins to be closed. This is accomplished by the development of the third part of the septum transversum; i.e., the *lateral closing fold*, extending from the mesocardia to the ventro-lateral body wall. By the eighth day, this closure is complete. In the meantime, the lungs have been developing in the portion of the peritoneal space which extends forward above the pericardial cavity. This space may be termed *pleural cavity,* and at this time (fifth day) the oblique lateral mesocardia have not yet entirely separated it anteriorly from the pericardial cavity beneath it; posteriorly also it still communicates with the general body cavity. Presently, however, with the further development of the lateral mesocardia and other parts, the opening between the pleural and pericardial cavities is closed, and a closure of that between the pleural and body cavities soon follows (tenth day). This latter is effected by the *pleuro-peritoneal septum,* which arises as an outgrowth from the sides of the œsophagus. The median pericardial cavity is thus bounded dorsally largely by the mesocardia, laterally and ventrally by the peritoneum of the body wall, and posteriorly chiefly by the median mass of the septum transversum.

THE ESTABLISHMENT OF THE DEFINITIVE PERICARDIUM

Eventually, however, the tissue upon the front of the median mass becomes thickened and splits into two sheets. The anterior sheet then becomes the posterior wall of the pericardium, the posterior sheet covers the face of the liver, and the general body cavity extends between them. At the same time, the latter cavity is also pushing forward beneath and at the sides of the present pericardium, and as it does so, it apparently splits the peritoneum of the body wall into two layers. The outer layer forms the peritoneum of the general body cavity in this region, and the inner layer constitutes the ventral and lateral wall of the pericardium proper. In this manner, the final pericardial wall or *definitive pericardium* of the adult bird comes to surround the heart as a relatively independent sac with a portion of the liver extending beneath it.
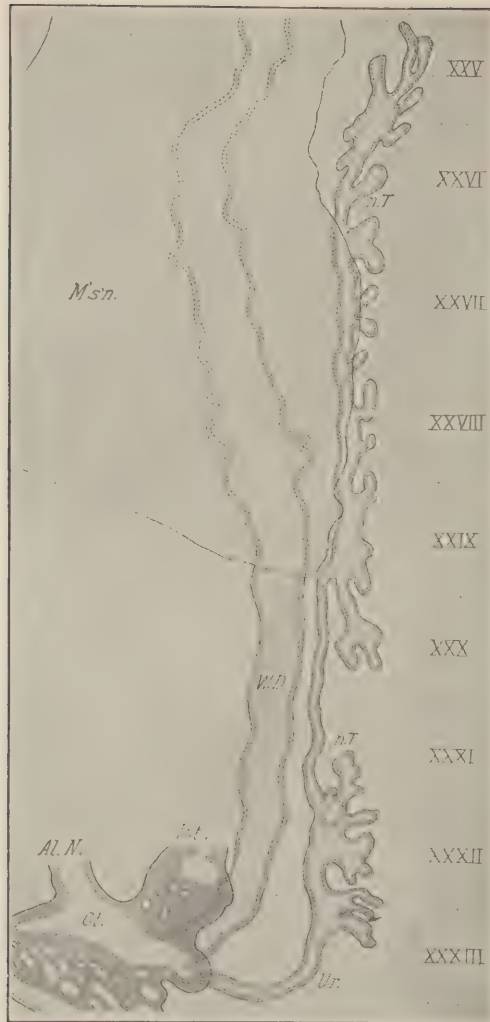
Fig. 213. — Profile reconstruction of the Wolffian duct and primordium of the meta-nephros of a Chick embryo of 6 days and 8 hours.   From Lillie (Development of the Chick).   After Schreiner.
    XXV to XXXIII, twenty-fifth to thirty-third somites.   *Al.N.* Neck of allantois.   *Cl.* Cloaca.   *Int.* Intestine.   *M's'n.* Mesonephros.   *n.T.* Nephrogenous tissue of the meta-nephros included within the dotted lines.   *W.D.* Wolffian duct.   *Ur.* Ureter.

THE URINOGENITAL SYSTEM

THE EXCRETORY SYSTEM

The Mesonephros. — During the fifth day, the increase in the number of the mesonephric tubules ceases, and the organ begins to function as a kidney. For a couple of days subsequent to this, however, the tubules continue to grow in length, thus greatly increasing the bulk of the organ. Degeneration begins about the eleventh day, and from then on, the metanephros aids in performing the excretory functions which it later entirely takes over.

The Metanephros. — At the end of the fourth day, the diverticulum (ureter) from the posterior end of the Wolffian duct had just appeared, and the nephrogenous tissue immediately behind the mesonephros had degenerated. During the fifth day, the above diverticulum, accompanied by the nephrogenous tissue posterior to the region of degeneration, grows forward somewhat, and begins to branch dichotomously (Fig. 213, representing a slightly later stage). Its position in this region is adjacent to the posterior cardinal vein, upon the median side of the latter and above the Wolffian duct. The accompanying nephrogenous tissue lies, in turn, adjacent to the median side of the diverticulum, so that the latter; i.e., the diverticulum, lies between the vein and the tissue. This nephrogenous tissue, which is in immediate contact with the diverticulum and its branches, is called the *inner zone*. Lastly this inner zone is covered on its median side by a layer of dense mesenchyme which differentiates in advance of the growing nephrogenous element and diverticulum. It is called the *outer zone* (Fig. 214).

During subsequent days, the posterior end of the mesonephric duct bearing the metanephric diverticulum (ureter) is drawn into the cloaca, and thus the ureter acquires an opening separate from that of the mesonephros (Fig. 213). The other end of the metanephric duct, with its inner and outer zones, meanwhile, grows still further forward till it reaches the region of the mesonephros, and then continues on dorsal to that organ, nearly to its anterior extremity. The inner zone of this tissue everywhere gives rise to the secreting tubules and glomeruli of the permanent kidney in a manner very similar to that described for the mesonephros. These tubules then connect with the dichotomous branches of the metanephric duct, which thus func-

tion as collecting tubules, while the duct itself becomes the ureter of the adult. Eventually the outer zone helps to form a connective tissue covering for the entire organ.

THE REPRODUCTIVE SYSTEM

The Gonads in the Male. — During the fourth day, it is impossible to distinguish sex. Upon the fifth day, however, the distinction becomes possible, at least in some cases, by the fact that in the female
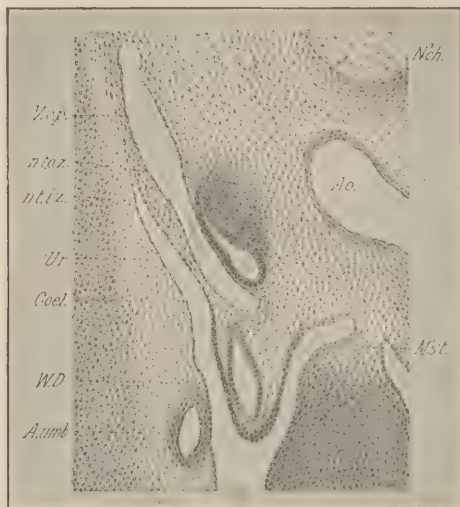
Fig. 214. — Transverse section through the ureter and metanephrogenous tissue of a five day Chick. From Lillie (Development of the Chick).

A.umb. Umbilical artery. *Cœl.* Cœlom. *M's't.* Mesentery. *n.t.i.z.* Inner zone of the nephrogenous tissue. *n.t.o.z.* Outer zone of the nephrogenous tissue. *Ur.* Ureter. *V.c.p.* Posterior cardinal vein. *W.D.* Wolffian duct.

the right gonad already appears less well developed than the left. In the male, which is to be considered first, however, there is no difference, and therefore the description of one gonad will suffice for both.

During the fifth day the primordial germ cells within the germinal epithelium begin to multiply, and this tissue is gradually drawn somewhat on to the ventro-median surface of the mesonephros. Meanwhile from the capsules of the Malpighian bodies of that organ, strands of cells begin to grow out through the loose mesenchyme to

the germinal epithelium. These strands are the *rete cords,* and are destined to form the *vasa efferentia* which help to connect the future tubules of the testis with the vas deferens (see below). At about this period also the germinal epithelium begins to send processes inward among the mesenchyme cells and the rete cords. These new strands of tissue of epithelial origin are the *sexual cords,* which contain many primordial germ cells (Figs. 215, 216). Up to this point the condition or the male gonad is virtually identical with that of the
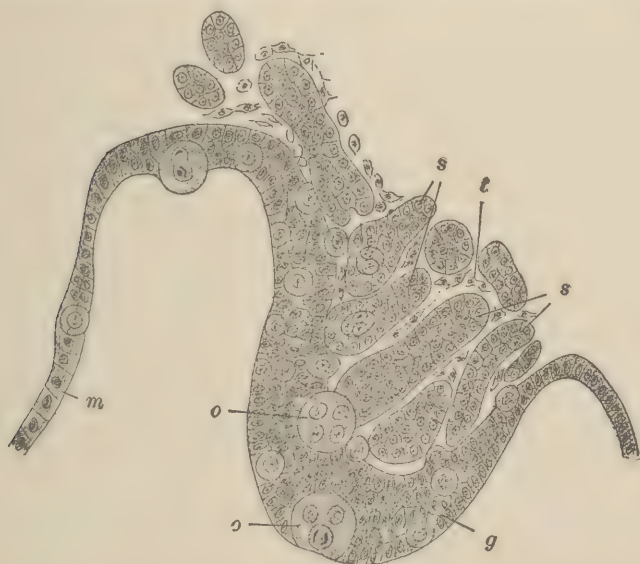
Fig. 215. — Section through the gonad of a chick, the middle of the fifth day, showing the sexual cords growing inward from the germinal epithelium. The connections of many of the cords with the epithelium have been cut across. From Kellicott (Chordate Development). After Semon.

g. Germinal epithelium. *m.* Epithelium of the mesentery (peritoneum). *o.* Primordial germ cells. *s.* Sexual cords. *t.* Connective-tissue stroma.

female. From now on, however, the former begins to be differentiated to form the adult *testis* in the following manner:

The sexual cords become separated from the epithelium, and increase in number so as to constitute the bulk of the organ (seventh day), while the rete cords are pressed to the side nearest the mesonephros. Presently also (eleventh day) the mesenchyme, which has been scanty, begins to increase among the sexual cords, forming the con-

nective tissue or *stroma*. Eventually it gives rise further to a layer, the *albuginea*, lying between these cords and the reduced sheet of epithelium which remains as the outer covering of the gonad. Meanwhile the sexual cords themselves (twentieth day) begin to acquire a lumen, and are thus transformed into the *seminiferous tubules*. The walls of the latter are composed of supporting cells which are lined
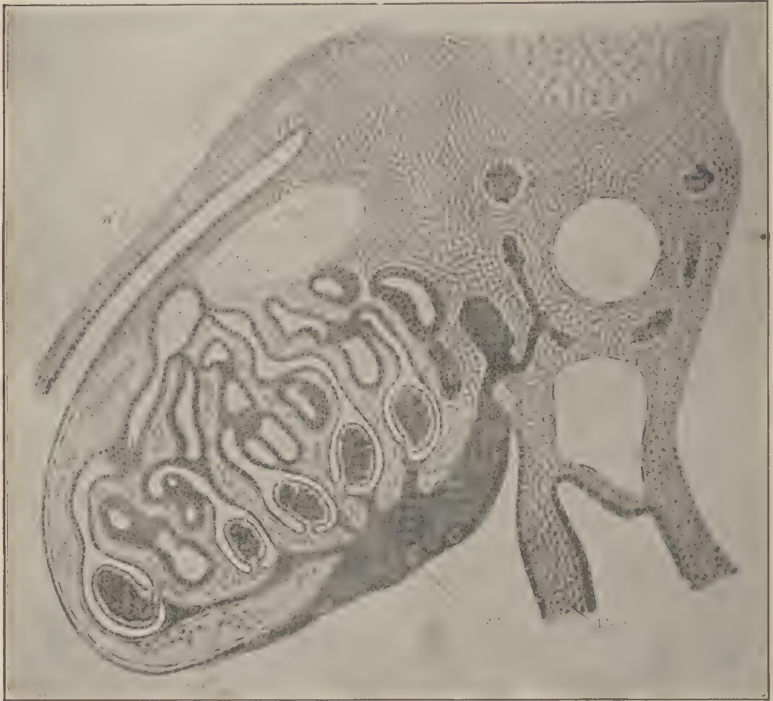
Fig. 216 — Cross-section through the genital primordium of *Limosa ægocephala*. From Lillie (Development of the Chick). After Hoffmann, from Felix and Bühler. The stage is about similar to that of a Chick embryo of 4½ days, and shows the rete cords extending from the Malpighian tubules to the germinal epithelium. The latter appears in the figure as a dark mass on the right ventral side of the mesonephros next to the mesentery. Three primordial germ cells (light colored) are visible in it.

Germ. Germinal epithelium. *Ms.t.* Mesentery. *S.C.* Rete cords. *V.* Posterior cardinal vein. *W.D.* Wolffian duct.

internally by the multiplying primordial germ cells. The latter may now be termed spermatogonia, from which arise in turn the spermatocytes and sperm (Fig. 217). It is to be noted in this connection that the spermatogonia, unlike the oögonia in the Bird, continue to divide throughout the sexual life of the individual. The ends of the

seminiferous tubules eventually become connected with the rete cords which, as indicated above, become the vasa efferentia. These in turn connect with the modified mesonephric tubules in the anterior or sexual half of that organ, which thus becomes the *epididymis*. The posterior and non-sexual portion of the mesonephros which remains becomes a vestige known as the *paradidymis*.

The Gonads in the Female. — Although differences in sex may be indicated by the disparity in the size of the gonads as early as the
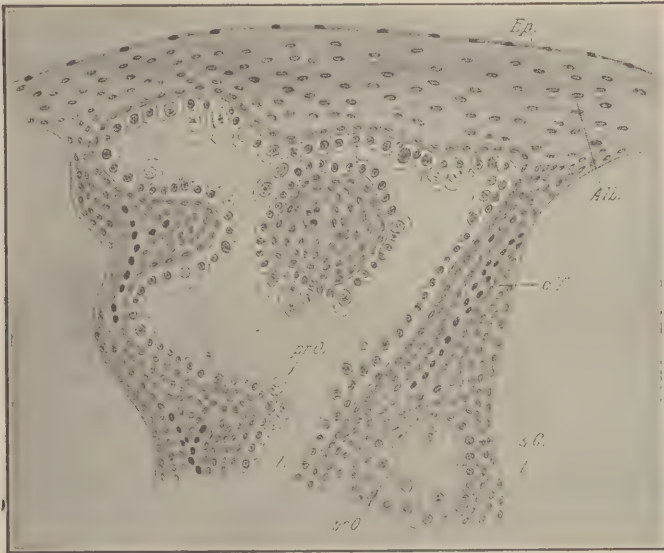
Fig. 217. — Cross-section through the periphery of the testis of a just hatched Chick. From Lillie (Development of the Chick). After Semon. The sexual cords have acquired a lumen, and the walls of the canals thus formed are lined within by the spermatogonia. Next to the latter come a layer of supporting or Sertoli cells. The connective tissue (stroma) lying between the sexual cords (now seminiferous tubules) connects at the periphery of the testis with the special layer of connective tissue (albuginea) which covers the entire organ beneath the thin outermost layer of germinal epithelium.

Alb. Albuginea. *c.T.* Connective tissue of the stroma, or septulae testis. *Ep.* Remains of the germinal epithelium now forming the outermost or serous covering of the testis. *l.* Lumen of the sexual cords. *pr.o.* Spermatogonia. *s.C.* Sexual cord, lined by supporting cells and spermatogonia.

fifth day, there is little else to distinguish male from female at this time. The description for the testis up to this point will, therefore, suffice for that of the left *ovary*. The right ovary need not be further considered, as it presently degenerates.

Subsequent to the fifth day, the following changes occur as the apparently indifferent gonad develops into that of the adult female. In the first place, the sexual cords together with such germ cells as have migrated into them eventually disappear, while the stroma, on the other hand, increases in abundance. At the same time, processes from the inner wall of the germinal epithelium once more push up into

Fig. 218. — Cross-section of the ovary of a fledgling of *Numenius arcuatus* 3–4 days old. The germinal epithelium is below. From Lillie (Development of the Chick). After Hoffmann. Note numerous oöcytes surrounded by a single layer of follicle cells. *s.c.* Sexual cords degenerating. *Germ. Ep.* Germinal epithelium producing ovigerous cords.

the stroma. They again carry primordial germ cells with them, and in this case are termed the *ovigerous cords*. Later, these cords break up into nests of cells each of which contains at least one oögonium; the remaining indifferent epithelial cells of each group then surround the oögonium to form its *follicle*. From this point on, the young

egg cell begins to grow, and it may, therefore, be termed an oöcyte (Fig. 218). This growth period is reached earlier by some ova than by others, but the oögonial or multiplication stage ceases for all about the time of hatching. The anterior portion of the mesonephros, which in the male forms the epididymis, remains as a minute rudiment, the *epoöphoron*. The paradidymis of the male is sometimes evident in the hen as a still smaller vestige, the *paroöphoron*.
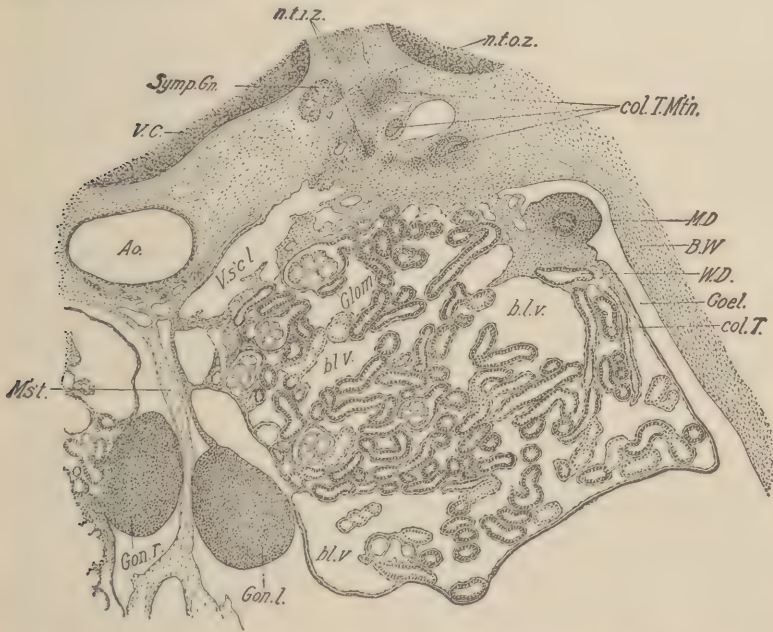
Fig. 218A. — Transverse section through the metanephros, mesonephros, gonads and neighboring parts of an 8-day Chick. From Lillie (Development of the Chick).

Ao. Aorta. *bl.v.* Blood vessels. *B.W.* Body-wall. *Cœl.* Cœlom. *col.T.* Collecting tubule of the mesonephros. *col.T.M't'n.* Collecting tubules of the metanephros. *Glom.* Gomerulus. *Gon.l.* Left gonad. *Gon.r.* Right gonad. *M.D.* Mullerian duct. *M's't.* Mesentery. *n.t.i.z.* Inner zone of nephrogenous tissue (metanephric). *n.t.o.z.* Outer zone of the nephrogenous tissue. *Symp.Gn.* Sympathetic ganglion of the twenty-first spinal ganglion. *V.C.* Centrum of vertebra. *V.s'c.l.* Left subcardinal vein. *W.D.* Wolffian duct.

The Gonoducts in the Male. — It has already been stated that in the male, the Wolffian ducts become the *vasa deferentia* or sperm ducts of the adult. They connect with the testes through the vasa efferentia and epididymis. Late in development, they become muscular and somewhat convoluted, with a dilation at their posterior extremities.

The Gonoducts in the Female. — As has been stated, the oviducts begin development on the fourth day as the tubal ridges, one on the lateral side of each mesonephros adjacent to the respective Wolffian duct. During the fifth day, a groove-like invagination develops along the anterior portion of each ridge, and the lips of the groove fuse with one another to make a tube open at its anterior end. This tube which is quite short, then grows backward independently between the remaining tissue of the ridge and the Wolffian duct (Fig. 218A).

Subsequent development is as follows: By the eighth day each duct has reached the cloaca, but does not open into it. At this time, there begins the atrophy of both ducts in the male and of the right duct in the female, accompanied in each case also by the degeneration of the remains of the respective tubal ridge. The left duct in the female, however, gradually enlarges and differentiates the infundibulum and glandular portions characteristic of the adult. It does not, however, effect an entrance into the cloaca until the hen is about six months old. (Lillie after Gasser.) It always remains attached to the body wall and the rudiments of the mesonephros by a ligament or mesentery-like fold.

THE ADRENALS

During the fifth day, the cortical substance, noted as arising upon the fourth day, increases in amount, and comes into relation with the Malpighian capsules. Throughout the sixth and seventh days, the above substance continues to increase, and upon the eighth day begins to be arranged in characteristic cords, surrounded by blood sinuses. Upon this day also the second element of the adrenals starts to develop; i.e., the "*medullary*" substance. It is derived from the cells of a sympathetic ganglion on the antero-dorsal side of the adrenal rudiments. This substance likewise ultimately takes the form of cords, which penetrate among those of the cortical material.

HATCHING

Upon the fourteenth day, the Chick turns so that its head is toward the large end of the shell, and the long axis of its body is parallel to that of the egg. From the seventeenth to the nineteenth days, the amniotic fluid decreases somewhat, the beak pierces the membrane of

the air chamber, and the intestine is retracted within the body. By the twentieth day, or slightly earlier, the Chick has begun slowly to breathe the air within the chamber. In correlation with this, the allantois starts to dry up, and the circulation within it to cease. The necessary changes in the pulmonary circulation also commence to occur, as described above. Meanwhile the yolk-sac, together with the dried remains of the allantois, which is attached to it, is entirely retracted within the body, and the opening of the umbilicus closes over.

It may now be noted that the *beak*, which has been developed by the cornification of epidermal cells around the margins of the mouth, has upon its dorsal surface a special horny point. This point is a temporary structure called the *egg tooth*. It is for the single purpose of piercing the membrane and shell; after this is done, it is lost. The shell is usually chipped toward the end of the twentieth day, and upon the twenty-first day, the Bird is hatched.

SUMMARY OF THE CONDITION AT THE END OF THE FIFTH DAY OF INCUBATION

I. THE EXTERNAL APPEARANCE

The *cervical flexure* has reached its maximum development, the *third visceral cleft* has closed, and the future *neck* is slightly indicated. The *limb buds* are beginning to appear jointed. The nasal apertures are separated into internal and external *nares* and the *beak* and *mandible* are just starting to form.

II. THE SKELETON

The definitive or *vertebral* segmentation of the *mesenchymal sheath* about the notochord and nerve cord has become more marked, while all the sclerotomal tissue is becoming membranous. These membranous condensations are especially evident in certain regions, representing parts of the *future vertebræ neural arches* and *costal processes*. Mesenchymal concentrations representing the *limb bones* and the parts of the *pectoral* and *pelvic girdles* are also visible. The various parts of the *primordial cartilaginous cranium* and *visceral skeleton* are discernible at this time as concentrations of mesenchyme about the head.

III. The Alimentary Tract

The Fore-gut Region. — The *third visceral cleft* closes, the *lung rudiments* have grown posteriorly somewhat through a mass of developing mesoderm, and faint indications of the *abdominal* and *cervical air sacs* may be present. The *glottis* is partly closed.

The *œsophagus* has continued to elongate, the *stomach* is slightly dilated, and a pouch representing the rudiment of the *gizzard* has appeared in connection with it. The *duodenal loop* is barely defined. The *liver* has continued to branch, and some of the branches have acquired lumens. The three *pancreatic diverticula* have also branched somewhat.

The Mid-gut Region. — The *mid-gut* or *small intestine* shows a slight dip (*duodeno-jejunal flexure*) into the yolk stalk to the point where it connects with the yolk sac. At its posterior limit, the rudiment of the *intestinal cœca* is visible.

The Hind-gut Region. — The *hind-gut* or *rectum* is not materially altered, but the laterally compressed walls of the posterior part of the cloaca have become fused.

IV. The Circulatory System

The Heart. — The *alterations* in the *relative positions* of the parts are nearly completed, as are also the *septa* within the heart. The *septum* of the *truncus arteriosus* has formed and that of the *bulbus* has started to develop.

The Arteries. — The portions of the *dorsal aortæ* between the third and fourth arches have begun to disappear, and the *left fourth arch* has also diminished in size. The *subclavian* arteries have become connected with the carotids and the *anterior mesenteric* and *cœliac* arteries are developed.

The Veins. — The right side of the *second venous ring* about the intestine has disappeared, so that in this region there is only a single vitelline trunk. Within the liver, the capillaries of the *ductus venosus* are continuing to develop, while the main channel is atrophying. The *mesenteric vein* has started to form.

The *subcardinals* have lost their original direct connections with the *posterior cardinals*, and have developed new ones through capillaries within each mesonephros. At the same time the subcardinals have

fused with one another anteriorly, and by means of the previous con-
nection with the *vena cava inferior,* have thus established a *renal portal
system.* The *subclavian* veins have started to develop from the pos-
terior cardinals.

V.   The Body Cavities

The ventral communication between the *pericardial* and *peritoneal*
cavities has begun to be closed by the development of the *lateral closing
folds* beneath the *lateral mesocardia.*

VI.   The Nervous System

In connection with the description of this system in the preceding
chapter, it was noted that there are few important developments
occurring in it on the fifth day. The following events, however, may
be mentioned as having taken place during this period. The *fourth
cranial nerves* have originated, and in connection with the ear the
rudiments of the *semicircular canals* have appeared. In the eye the
mesenchymal part of the *pecten* is increasing, while the lips of the
choroid fissure are beginning to overgrow it.

VII.   The Urinogenital System

The Excretory System. — The *mesonephric tubules* have ceased to
increase in number, but are continuing to grow in length, and the
organ has begun to function. The *metanephric diverticulum,* accom-
panied by its *nephrogenous tissues* or *inner zone,* has grown forward
and begun to branch, while about the latter the *outer zone* is developing
from mesenchyme.

The Genital System. — The *primordial germ cells* have begun to
multiply within the *germinal epithelium* and the *rete* and *sexual cords*
have started to develop. The male and female gonads are similar
except for occasional differences in size between the right and left
organs in the female. In both sexes, the *oviducts* are present as
small tubes growing toward the cloaca.

VIII.   The Adrenals

The *cortical substance* of the adrenals increases in amount, and comes
into relation with the Malpighian capsules.

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PART V

THE MAMMAL

THE EARLY DEVELOPMENT OF THE MAMMAL AND ITS EMBRYONIC APPENDAGES

The early development of the Mammal is of particular interest because during this period, as is so often the case, there appear various features which suggest the previous history of the group. Furthermore, a study of these earlier stages, especially from the comparative standpoint, helps to make clear the character and probable evolution of those membranes and appendages which are connected with the peculiar mammalian relationship of mother and fœtus. The latter, moreover, is perhaps the most significant feature which distinguishes the development of the Mammals from that of the Birds and Reptiles (Sauropsids), and is therefore the point of chief importance for the general student to comprehend. It will be found desirable, as previously, to begin with a description of the structure and functions of the reproductive organs of the adult male and female, in this case particularly those of the latter.

THE REPRODUCTIVE ORGANS OF THE ADULT

The Male

The Testes and their Ducts. — In the adult male Mammal there are normally two testes. These organs may be retained permanently within the body of the animal, as in the case of the Elephant; more commonly, however, they pass out of the body during development, and are contained either in two sacs, or in two chambers of a single one, the *scrotal sac* or *scrotum*. In some cases, as among Rodents, an intermediate condition occurs in which the testes descend into the scrotum only during intervals of sexual activity. Each testis consists of the usual seminiferous tubules, embedded in connective tissue and leading by way of vasa efferentia to the respective vas deferens.

Accessory Organs. — In the Mammal there are, in addition to the testes and other parts just noted, certain accessory organs connected with the more distal parts of the genital tract. These are the *prostate glands, Cowper's glands,* and, in some animals (e.g., in Man), the *seminal vesicles.* The function of the glands is to furnish a suitable medium for the existence of the sperm after it leaves the organs of the male. The vesicles, on the other hand, presumably assist both in the secretion of additional fluids and in storing the combined sexual products or *semen* previous to its ejaculation. Finally, there is in the male Mammal a *penis.* This has a single duct, the *urethra,* which serves to discharge urine, and also to introduce the semen into the genital tract of the female.

THE FEMALE

The Ovary. — In the female Mammal there is a single pair of ovaries, and, as in the other forms studied, these organs are contained within the body cavity and suspended from its wall by a mesovarium. The ovaries are whitish ovoid objects, varying in size in different animals, but always relatively small. Thus in the Human Being, for example, each ovary is about 3–4 cm. long, and from 2–3 cm. wide. Fundamentally, their internal structure is similar to that already described in the Bird.

The Genital Tract.

The Oviducts. — As in the Bird, the ovaries are not immediately connected with the Müllerian ducts or oviducts. The latter, sometimes known as the *Fallopian tubes,* are, however, provided as usual with a typical fimbriated funnel, or *infundibulum,* which serves to embrace the ovary when an ovum is discharged. The oviducts themselves consist of walls made up as follows: On the outside is the *serous membrane,* next to that a layer of more or less mingled *longitudinal and circular muscles,* then a sheet of vascular connective tissue known as the *mucous layer,* and finally a coat of *ciliated epithelium.* From the infundibula these ducts pass posteriorly and presently unite.

The Uterus and Vagina. — In the general region of their union the walls become much thicker, due particularly to an increase in the muscular and mucous tissue, the latter now containing lymph spaces and many *glands.* The portion of each tube thus characterized is

known as a *uterus*. It is to be noted that this change in the nature
of the ducts may occur entirely proximal to the actual point of union
(Rodents); in this case there are two uteri, and the condition is
known as *uterus duplex*. On the other hand the uterine character of
the tubes may exist both above and below the region of union
(Carnivora and Ungulata), and in such instances the condition is
termed *uterus bicornis*. Finally, the entire uterine portion of the
ducts may be fused to form a single cavity (Primates), a condition
called *uterus simplex*.

Beyond the uterus, or uteri, as the case may be, there is a single
passage leading to the exterior, known as the *vagina*. At the external
end of the latter there are certain rudiments homologous with the
penis of the male.

THE DEVELOPMENT OF THE OVUM UP TO SEGMENTATION

OÖGENESIS

The Oögonia. — The embryonic ovary of the Mammal contains the
usual primordial germ cells which, as in the lower Vertebrates, have
probably migrated thither from the walls of the gut. At first these
cells lie chiefly in the outer epithelium or cortex of the ovary. Pres-
ently, however, this epithelium thickens, and is penetrated by the
ovarian connective tissue element, or stroma, thus becoming cut up
into strands or cords. These are the *ovigerous cords* (egg tubes),
similar to those described in the Chick, but in this instance often
called the *cords of Pflüger*.[1] As in the Bird, they contain both the
female germ cells, or oögonia, and numerous epithelial cells as well.
In the Mammal, however, the two types of cells are not easily dis-
tinguishable from one another, and it is quite possible that some germ
cells may arise *in situ* from indifferent cells of Pflüger. During this
period multiplication of all the cells goes on rapidly.

At some time before the birth of the animal in which the ovary
is contained, however, multiplication of the oögonia ceases. The cells
are then arranged in nests or groups, each of which contains a single
oögonium, the remaining epithelial cells in the group being destined

[1] In some instances (e.g., Man), the arrangement of the cells in cords is said
not to occur (Felix, Chap. XIX in Keibel and Mall, *Human Embryology*)

to form the follicle. The young ovum now enters upon the growth period as an *oöcyte*.

The Oöcyte and the Graafian Follicle. — At about this time, the epithelial cells referred to begin to become arranged about the young ovum to form the highly characteristic mammalian or *Graafian follicle*. At first they constitute a thin flat layer only one cell thick, but soon multiply so as to form a mass of cells about the growing oöcyte. In one side of this mass there then appears a space, the
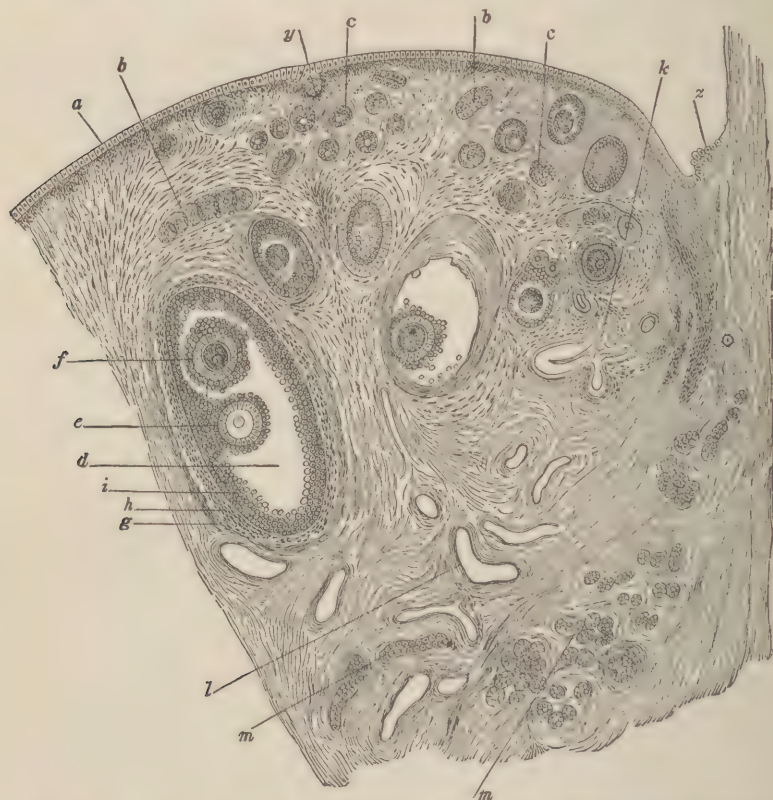
Fig. 219. — Section through part of the ovary of a Dog. From Kellicott (Chordate Development). After Waldeyer

a. "Germinal epithelium." *b.* Ovigerous cords. *c.* Small ovarian follicles. *d.* Older ovarian follicle. *e.* Ovum surrounded and attached to wall of follicle by discus proligerus. The corona radiata is visible but is not labeled. *f.* Second ovum in follicle with e. (Only rarely are two ova thus found in a single follicle.) *g.* Outer layer of follicular capsule. *h.* Inner layer of follicular capsule. *i.* Membrana granulosa. *k.* Collapsed, degenerating follicle. *l.* Blood-vessels. *m.* Sections through tubes of the parovarium. *y.* Involuted portion of superficial epithelium. *z.* Transition to peritoneal epithelium.

follicular cavity, which gradually enlarges and extends around the sides of the oöcyte. These extensions, however, never quite meet. Thus the oöcyte, still closely surrounded by several layers of cells, is suspended within the follicular cavity, which becomes filled by a fluid, the *liquor folliculi.* Meantime, the outside of the entire follicle has become covered by a capsule (*follicular capsule*), formed externally of connective tissue and internally of cells, blood vessels, and nerves.

The various layers and parts of the entire Graafian follicle may now be named, as follows: Beginning on the outside there is the follicular capsule with its inner and outer layer. Just within this, and bounding the follicular cavity, there are a few layers of the follicular cells forming the *basement membrane,* or *membrana granulosa.* Upon the side of the ovum where the cavity has not extended, a neck of cells reaches from this membrane to those cells which immediately surround the oöcyte. Thus the latter is attached to the inner wall of the follicle by this neck, which, together with the more peripheral of the cells immediately surrounding the ovum, is termed the *discus proligerus.* Those of the immediately surrounding cells which have remained closest about the egg are now gradually elongated at right angles to the surface of the latter, and are eventually known as the *corona radiata* (Fig. 219). This brings us to the actual egg and its membrane.

The Mature Ovum

The mature ovum in all placental Mammals [2] is relatively minute, though naturally varying in size in different animals. Thus that of the Dog measures about 0.18 mm. in diameter, that of Man 0.22 mm., and that of the Cat 0.15 mm. The reason for this minute size is the fact that mammalian eggs are virtually without yolk (alecithal). They consist of a central region of opaque endoplasm surrounded by a thin layer of exoplasm, and within the former is a relatively large nucleus (germinal vesicle), somewhat excentrically placed.

The ovum apparently does not possess any true vitelline membrane. It is surrounded, however, by a thick transparent substance which is presumably chorionic; i.e., is secreted by the cells of the follicle. This layer, though clear, frequently appears to be perforated by minute

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[2] It will suffice to state at this point that the term *placental Mammal* includes the vast majority of the group. Its exact significance will be fully described in the section on the yolk-sac, allantois, and placenta (see below).

canals through which processes of the follicular cells reach the egg to nourish it. It is, therefore, known either as the *zona pellucida* or the *zona radiata*. There is usually a slight space between this zone and the protoplasm of the egg, and though there may be no vitelline membrane this space is known as the *perivitelline space* (Fig. 220).
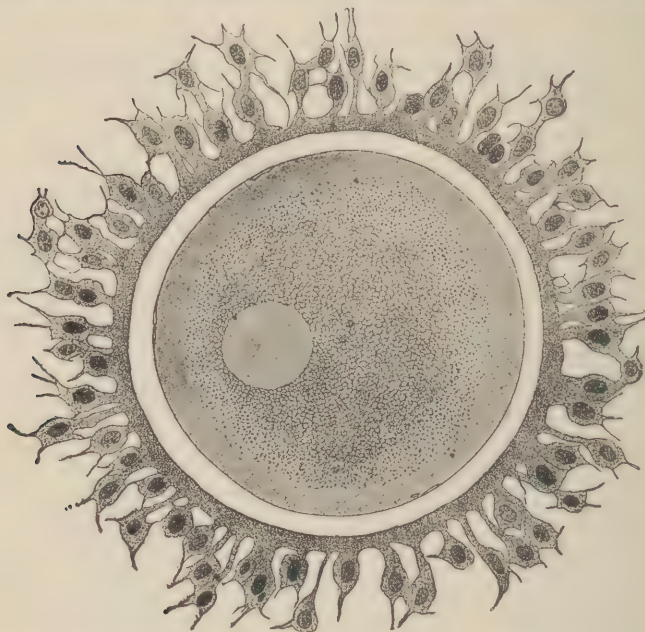
Fig. 220.—Fully grown Human oöcyte just removed from the ovary. Outside the oöcyte are the clear zona pellucida and the follicular epithelium (corona radiata). The perivitelline space in this instance is not apparent. The central part of the oöcyte contains deutoplasmic bodies and the eccentric nucleus (germinal vesicle). Superficially there is a well-marked exoplasm, or cortical layer. From Waldeyer (Hertwig's Hand-buch, etc.).

OVULATION AND THE CORPUS LUTEUM

Ovulation. — As a Graafian follicle and its ovum matures, it is gradually brought to the surface of the ovary. At the same time one side of the follicle becomes thin in connection with the formation of a *cicatrix*, as in the Chick. As complete maturity is reached, the discus proligerus is broken and the ovum floats freely in the liquor folliculi. In most animals rupture of the follicle then occurs spontaneously, and

its contents is received by the infundibulum of the oviduct. In a few forms; e.g., the Rabbit and Cat, the breaking of the ripe follicle does not usually occur spontaneously, but only following copulation with the male (*coitus*). The liberation of an ovum may or may not take place in both ovaries at once, and there may or may not be more than one follicle ready for discharge in the same ovary at approximately the same time. These variations, moreover, may occur normally in the same species of animal. In Mammals which ordinarily produce a litter of young, however, the discharge of several ova at once is of course the usual thing.

The Corpus Luteum. — Following the discharge of the ovum, the empty follicle undergoes a peculiar transformation. It presently becomes filled with large cells containing a special coloring matter. This matter is yellow in Man, and is therefore known as *lutein,* while the follicle when thus transformed is called the *corpus luteum*. The origin of these lutein-filled cells has been in some doubt, but it appears most likely that they are derived from the membrana granulosa.

The Significance of the Corpus Luteum. — If pregnancy occurs, it has been found that the corpus luteum persists during perhaps half the period of gestation. If pregnancy does not occur, however, the corpus luteum atrophies and disappears within a few days. These facts have led to much speculation and interest in regard to the function of these bodies. It now seems fairly certain, however, that the corpus luteum produces an internal secretion containing a *hormone* whose object is twofold. First, it causes a modification of the uterine wall such that the ovum, if fertilized, will become fixed thereto as described below. This statement is made on the ground of certain experiments tending to show that if the corpus luteum be removed, fixation of the fertilized ovum will not occur. Second, during the early stages of pregnancy this hormone helps in some way to " maintain the raised nutrition of the uterus " (Marshall) necessary for the development of the embryo.

This latter conclusion, moreover, has a double basis: first, experiment appears to show that if the corpus luteum be removed in the early stages of pregnancy, abortion at once follows; secondly, there is the fact already noted, that if fertilization, and hence pregnancy, has failed to occur, the corpus luteum soon disappears.

The above facts seem to indicate the probable functions of the corpus luteum. Nevertheless, the problem as to what mechanism causes this

body to persist when needed and disappear when not needed, still remains to be solved. Presumably there is a reciprocal action involved between the corpus luteal tissue and the condition which it helps to bring about. Thus the general physiological changes throughout the system induced by pregnancy favor the growth and persistence of the corpus luteum. At the same time the persistence of this body produces something which is necessary in order that pregnancy itself may continue during its early stages.

THE SEXUAL CYCLE IN MAMMALS

It is well known that like many other animals, Mammals are capable of breeding only during certain periods or seasons. Among the Mammals, moreover, these periods are far more marked in the female than in the male. In the former sex they are also apparently related to some extent to the process of ovulation. On that account, therefore, it seems desirable to give a brief discussion of this subject at this time.

The Sexual Cycle in the Female. — In all placental Mammals which have been carefully studied, it is known that during sexual life the walls of the uterus suffer a series of periodic changes, interrupted only by pregnancy. These periods consist essentially of (1) a time of quiescence; (2) a time of construction during which the mucous layer of the uterine wall is congested with blood; (3) a time of destruction during which more or less of this blood is lost, together with some superficial parts of the wall itself; and (4) a time of repair during which the epithelium and mucous layers are restored to their quiescent condition.

These periods are further named as follows:

(1) Period of quiescence, $\begin{cases} \text{if short } \textit{diœstrum.} \\ \text{if long } \textit{anœstrum.} \end{cases}$

(2) Period of construction, $\Big\}$ *proœstrum.*
(3) Period of destruction,

(4) Period of repair, *œstrus.*[3]

The length of these periods varies greatly in different animals. Thus in the Rat and Guinea Pig, the whole cycle, including a diœstrum, is

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[3] The period of repair is sometimes divided into *œstrus* and *metœstrum*, the latter being a very short interval during which the repairing processes slow down and cease.

about ten days, while in certain Sheep it is three weeks. In all cases the length of the cycle also varies somewhat with temperature and food. Furthermore, not only does the length of the total cycle vary, but the relative lengths of its periods are also somewhat different even in the same animals at different times. In general, however, each has an approximately fixed number of days for its duration; e.g., in the Rat the period of prœstrum is from one to four days, and the period of œstrus about the same. It is to be noted here that in most animals it is only during the latter interval that the female is willing to receive the male, and is capable of conception. Thus among breeders the period of œstrus is spoken of as the period of " heat." [4]

This period of " heat " occurs only during the so-called " breeding season " of the animal in question, and with respect to this season there is again a large amount of well-known variation. Thus the season differs as regards its length, the number of times it may recur within a year, and the number of periods of heat included within a single breeding season. Some species of Mammals are able to breed the year round, while others are capable of reproduction only during a short interval occurring at a definite time of year. In the latter case, œstrus may occur only once during the season, or, as always in the former instance, the entire cycle may be repeated several times. In the event of such repetition the breeding season naturally tends to be longer, while at the same time the number of opportunities for fruitful copulation is increased. When the close of the season is reached, if pregnancy has not occurred the last diœstrum simply lengthens into an anœstrum, which continues until another prœstrum initiates the next breeding period. As noted, this may not occur again for another year, as in some Deer, or it may return again in six months, as in many Dogs, and in some Sheep. Outside of the Primates, which will be considered presently, the climax in the shortening of the anœstrum is reached in a certain race of Australian Merino Sheep, in which it practically disappears. That is, in this race there is a continuous succession of all four periods, the short diœstrum being the only time of quiescence. Thus these animals are able to breed the year round.[5]

This brings us to a consideration of the condition in the Primates, including Man. Here the period of prœstrum is termed the time of

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[4] Sometimes prœstrum is also erroneously included under this term.

[5] In the absence of pregnancy, it appears that in these animals œstrus or heat occurs about once in two months.

menstruation, and occurs normally about once in four weeks. The uterine changes occurring at this period appear to be somewhat more thorough-going than in some of the lower animals, and there is usually a more copious external discharge. This and other minor differences have led some to assert that the two sets of phenomena are not homologous, but there now seems little doubt that they are fundamentally the same. Furthermore, in the Primates, as in the race of Sheep referred to above, the succession of the periods is continuous. In spite of this, Monkeys seem to have retained a certain season of the year when breeding is more usual, and there may also remain a slight tendency of this sort in Man, especially among primitive peoples. With him, however, breeding is possible at any season, and copulation, though perhaps more likely to be fruitful during œstrus, may be so at any time between the menstrual periods.

The Function and Cause of the Female Cycle and its Relation to Ovulation. — Finally there remain the two questions (1) as to the physiological meaning of the sexual cycle, and (2) as to its cause, and relation to ovulation. These are problems concerning whose solution the best authorities differ. Since in so brief an account it is impossible to give a complete discussion of the matter, it appears most satisfactory merely to present in each case one of the best substantiated views. These also are views which are in harmony with the homology which has been assumed to exist between the procestrum and menstruation.

First, as regards the function of the procestrum or menstruation, it is held probable that it is a kind of preparation of the uterine wall which enables it to receive the fertilized ovum. Whatever the real nature of this change, it is of course previous and in addition to any further preparation, such as has been assumed above to be effected by secretions from the corpus luteum. This follows from the fact that it appears to occur in each cycle before the corpus luteum of that cycle is present.

Second, concerning the cause of the procestrum and its relation to ovulation, the following may be said: It seems most probable that the former process is brought about by the presence in the blood of some hormone which apparently originates from the ovary. This is strongly indicated by repeated evidences that removal of the ovaries causes menstruation to cease. Furthermore, it is a fact that in point of time the procestrum quite generally immediately precedes the process of ovulation. This relationship of the two phenomena suggests, there-

fore, that the enhanced metabolic activity of the ovaries which accompanies the later stages of ripening follicles may result in an increase of the hormones, which in turn produce the proœstrum. If this is so, the frequent temporal relationship of the latter process and ovulation is of course explained.

The Sexual Cycle in the Male. — As regards the male among Mammals, it is found that here also there is a tendency toward cycles of sexual activity. This phenomenon, however, is not so common as among the females, or among the males of lower forms. In those species of Mammals in which the male does experience special periods of heightened sexual desire, however, these normally coincide with the breeding season of the female, and are known as the *rutting periods*. At such times the males may develop very special secondary sexual characters, such as the antlers of the buck deer, as well as great irritability and desire for combat with other males. On the other hand, the males of many Mammals have no such special periods of sex activity. Instead, they are apparently able to breed at any time, even though the females of their kind will only receive them at certain seasons.

With this understanding concerning the nature of the sexual cycle and its relation to ovulation and sexual activity, we are now prepared to return to the history of the ovum.

Maturation and Fertilization

Although in Mammals the first maturation division often occurs before ovulation and fertilization, the second occurs afterward. Hence it has seemed best to mention both divisions in connection with the latter phenomenon.

The First Maturation Division. — At some time during the growth of the oöcyte, the preliminary stages of maturation are completed without any peculiarity of note. The first polar spindle is then formed, and usually a short time before ovulation the first polar body is given off. In the latter connection the only feature to be noted as peculiar to Mammals is the fact that this polar body is normally relatively large; i.e., often as much as one fourth the diameter of the ovum itself, and in abnormal cases sometimes equal to the latter. The fate of these exceptionally large bodies is not known. After the extrusion of the first polar body, the spindle for the second is formed and moves

into position for division. The completion of the process is then inhibited while ovulation and fertilization occur.

Fertilization. — Sperm introduced into the vagina of the Mammal rapidly make their way into the uterus and up the oviducts. A few hours or even less suffices for them to reach the upper ends of these ducts where the actual process of fertilization usually takes place. Though ovulation may not occur so that an ovum is present just when the sperm arrive, it is known that both products are able to exist in this locality in many cases for a week or more. Then when the
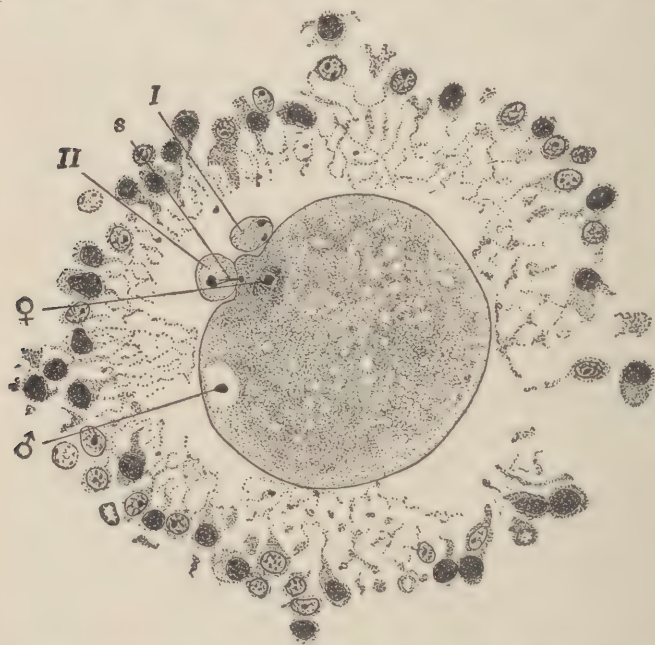
Fig. 221. — Reconstruction of four sections through the fertilized ovum of the Cat. From Longley (combined from two figures). No zona pellucida is visible in these sections. The corona radiata is disintegrating.

s. Remains of second polar spindle. *I*. First polar body. *II*. Second polar body. ♂. Sperm nucleus. ♀. Egg nucleus.

sperm and ovum do meet the latter is at once surrounded by the male germ cells. These make their way through the corona radiata and zona radiata which still cover the ovum, and one of them enters it; the others are normally prevented from doing so, apparently by some change in the cortex of the egg. In many cases, only the head and

middle piece of the sperm enter, but in others (Mouse), the entire spermatozoön is taken in; when this does occur, however, the tail soon degenerates. The head of the sperm next forms the sperm nucleus in the usual manner.

The Second Maturation Division. — After the entrance of the sperm and while the nucleus of the latter is forming, the second maturation division of the ovum is completed, resulting in a second polar body, usually smaller than the first. This is soon followed by the union of the sperm and egg nuclei, and the process of fertilization is complete (Fig. 221).

SEGMENTATION, GASTRULATION, AMNION FORMA- TION, AND THE PRIMITIVE STREAK

SEGMENTATION

The Type of Cleavage. — Segmentation in the placental Mammals is total, as might be expected from the virtual absence of yolk. The arrangement and behavior of the cells, however, is quite different from
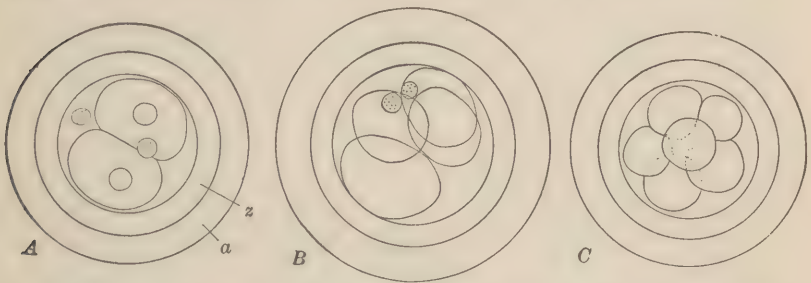
Fig. 222. — Cleavage of the ovum of the Rabbit. From Kellicott (Chordate Develop- ment). After Assheton. A. Two-cell stage, twenty-four hours after coitus, showing the two polar bodies separated. B. Four-cell stage, twenty-five and one-half hours after coitus. C. Eight-cell stage.
  a. Albumenous layer derived from the wall of the oviduct. z. Zona radiata.

that observed in the first yolkless form which was studied; i.e., Amphioxus. The reason for this is apparently due to the fact that the egg of a Mammal is almost certainly only secondarily without yolk. The evidence for this assumption will become more and more obvious in the course of this chapter, but a couple of the more striking proofs may be indicated here. Thus as will appear, the embryos of the

primitive non-placental Mammals known as Monotremes possess both yolk-sac and yolk, while all the placental Mammals retain the sac, though it is empty. Secondly, there are the origin of the embryo from what amounts to a blastoderm, the method of gastrulation, and other features all characteristics of large-yolked forms. We may now proceed to the actual method of segmentation.

The Blastocyst. — Cleavage, though total, is irregular from the start (Fig. 222). The result is the formation of a spherical mass of cells known as the *morula* in which the cells are of two types. On the
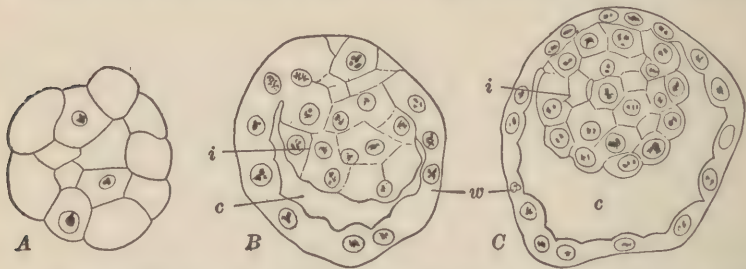
Fig. 223. — Morula and early blastodermic vesicles of the Rabbit. From Kellicott (Chordate Development). After Assheton. The zona radiata and albumenous layer are not shown. A. Section through morula stage, forty-seven hours after coitus. B. Section through very young vesicle, eighty hours after coitus. Taken from uterus; ordinarily the ova have not reached the uterus at this age. C. Section through more advanced vesicle, eighty-three hours after coitus. Taken from uterus.
c. Cavity of blastodermic vesicle. *i.* Inner cell mass. *w.* Wall of blastodermic vesicle (subzonal layer, later called trophoblast).

outside they are at first cubical, but soon assume the form of a flattened epithelium. Inasmuch as they are covered for a time by the zona radiata, they are known as the *subzonal layer*. The cells on the inside, on the other hand, are spherical and are called the *inner cell mass*. Presently, vacuoles appear on one side of this mass, beneath it and the subzonal layer. These run together and increase until more than half of the morula is occupied by a fluid-filled cavity. On the other side, the inner mass hangs from the wall like a suspended drop (Fig. 223). The morula has now become a *blastodermic vesicle* or *blastocyst*, which corresponds in a general way to the blastula of lower forms. Hence the cavity may be termed the *blastocœl* or *subgerminal cavity,* while the fluid within it occupies the place of the yolk. Finally, as subsequent development shows, the inner cell mass lying above the fluid virtually plays the part of a *blastoderm* (Fig. 224).

These steps usually occur while the ovum is passing down the oviduct, and are completed by the time it reaches the uterus. The time required for this passage varies much in different animals, but is ordinarily considerable; e.g., about four days in the Rabbit, and eight or ten days in the Dog.

Having arrived in the uterus, the blastocyst now begins to enlarge through the multiplication and flattening of the cells of the subzonal layer (Fig. 224). There is considerable variation in the size and
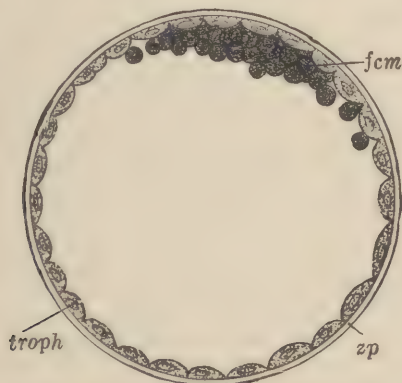
Fig. 224. — Section through the fully formed blastodermic vesicle of the Rabbit. From Quain's Anatomy, after Van Beneden.

fcm. Granular cells of the inner cell mass. *troph.* Trophoblast. *zp.* Zona pellucida.

shape which is reached in this manner. Thus in the Rabbit, the vesicle after three days in the uterus becomes ovoidal, measuring about 4.5 x 3.5 mm. In Ungulates, on the other hand, it becomes very long and tapering, that of a twelve-day Sheep being about 20 cm. long and 1–2 mm. in diameter. In all cases, however, the inner cell mass remains very small, and in instances where the vesicle is elongated, as in the Sheep, the mass is attached about midway between its ends.

GASTRULATION

As in the other forms studied, this term is here used in a strict sense, including only the formation of the *ectoderm* and *endoderm*. In most Mammals, the latter appears to arise by a splitting off of cells from the ventral side of the inner cell mass (i.e., the blastoderm). These cells then multiply and spread round the inside of the vesicle.

In most forms they completely line it eventually, just as they finally line the yolk-sac of the Bird. Thus this cavity with its lining is from now on termed the *yolk-sac* of the Mammal, though it contains no yolk, and, in a few cases, such as the Guinea Pig, the endodermal lining is not completed ventrally. Moreover, among the Primates the vesicle
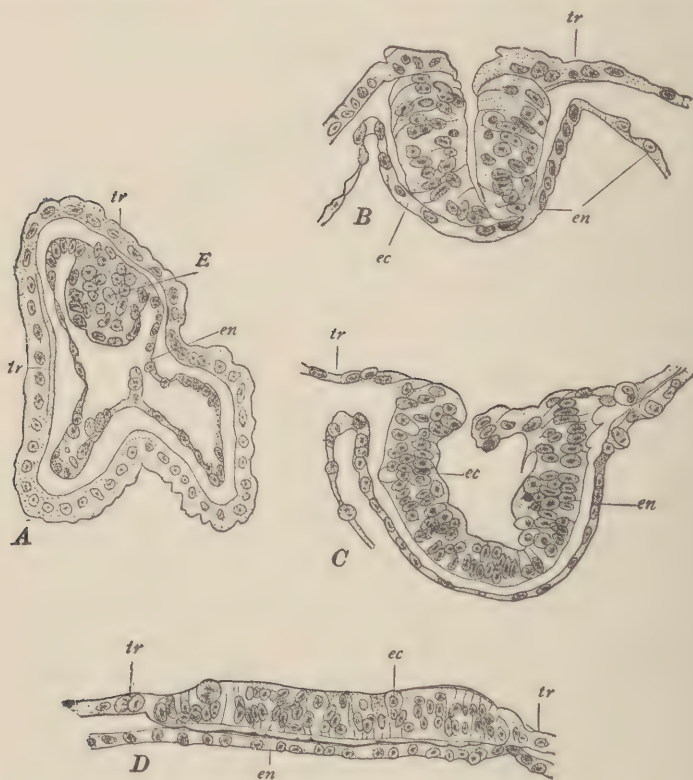
Fig. 225. — Sections through four stages in the early development of the Insectivor (*Tupaija javanica*). From Hubrecht. A. Blastodermic vesicle completely closed; endoderm still continuous with the embryonic ectoderm. B.C. Embryonic ectoderm split and folding out upon the surface of the vesicle, pushing away the trophoblast cells. D. Embryonic ectoderm forming a flat disc on the surface of the blastodermic vesicle. *E*. Inner cells mass, now embryonic knob. *ec*. Embryonic ectoderm. *en*. Endoderm. *tr*. Trophoblast.

enlarges so rapidly that the endoderm cannot keep up with it. Instead, therefore, it forms a small yolk-sac suspended within the vesicle. In any case the remainder of the inner cell mass together with the original

subzonal layer may now be termed the ectoderm. This ectoderm is then further divided into that which composes the remaining inner cell mass, now termed the *embryonic knob,* and that which composes the subzonal layer, now termed the *trophoblast.* It is to be noted that the latter completely encloses, for a time at least, the embryonic knob and the yolk-sac. Hence though originating differently, it occupies the same position as the chorionic ectoderm of the Chick (Fig. 225, *A*). In fact, with the mesoderm which in some cases later comes to line it, this layer constitutes the *chorion* of the Mammal.

It is to be clearly understood that the process of gastrulation which has just been described is entirely one of delamination and proliferation; there is apparently no involution, invagination, nor epiboly, and hence also no concrescence. Consequently, it is not surprising that there is no well marked *blastopore,* at least in connection with the actual process of endoderm formation. Later, as in the Chick, a primitive streak arises as a thickening in the ectoderm, and again as in the Bird, parts of this streak are interpreted by many as the homologue of a blastopore. This will be discussed further when the origin of the primitive streak is described.

IMPLANTATION

By the time the stage described above has been reached, and sometimes somewhat earlier, the blastocyst has become attached to the uterine wall. This process is known as *implantation,* and there are several methods by which it is brought about. It will be best, however, to postpone their detailed discussion until the description of the placenta is taken up. Suffice it to say at this point that it is brought about largely by the activity of the trophoblast, aided by certain changes in the uterine wall itself.

THE AMNION

There are two chief methods by which the amnion is formed in the Mammal:

I. **The First Method of Amnion Formation.** — This method may be defined briefly as the method of amnion formation by folds. The first step in this method involves the transformation of the embryonic knob (ectoderm) into a flattened plate overlying the endoderm, the

two layers being virtually homologous with the similar ones of the avian blastoderm. This flattening is accomplished, however, by two different processes. Thus though subsequent development of the amnion itself is similar, it is convenient upon the basis of the above differences in the initial stages to describe method I under two headings, Type (*a*) and Type (*b*).

Method I Type (*a*). — This type is illustrated by one of the Insectivors, *Tupaija* (Fig. 225); in this animal a depression appears in the top of the embryonic knob, and extends well down into it. The bottom of the depression then rises to the surface, and the edges are at
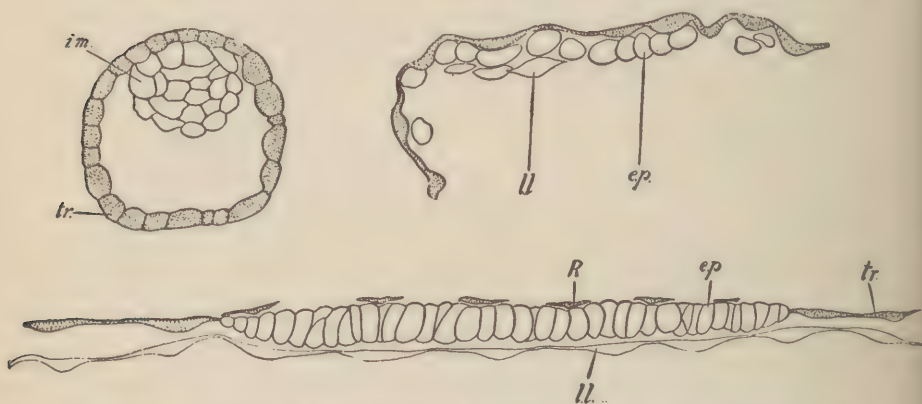
Fig. **226.** — Formation of the amnion in the Rabbit (*Lepus*). From Jenkinson (Vertebrate Embryology). After Assheton.

i.m. Inner cell mass. *l.l.* Lower layer (i.e. endoderm). *e.p.* Embryonic plate (i.e. blastodermal ectoderm). *R.* Cells of Rauber. *tr.* Trophoblast.

the same time pushed apart. As this occurs the trophoblast cells above are broken and scattered. Thus the ectodermal plate of the blastoderm so formed comes to lie directly on the surface of the blastocyst.

Method I Type (*b*). — In this type, of which the Rabbit (Fig. 226) is an example, the process is simpler, for here the knob merely flattens without the occurrence of any previous depression. In this instance after the flattening is completed, scattered trophoblast cells may remain for a time over the blastoderm, and are known as the cells of Rauber; these however, soon disappear.

Subsequent Stages of Method I Types (*a*) *and* (*b*). — As suggested above it will now appear that the later stages of types (*a*) and (*b*) are virtually alike. Before they are described, however, it should
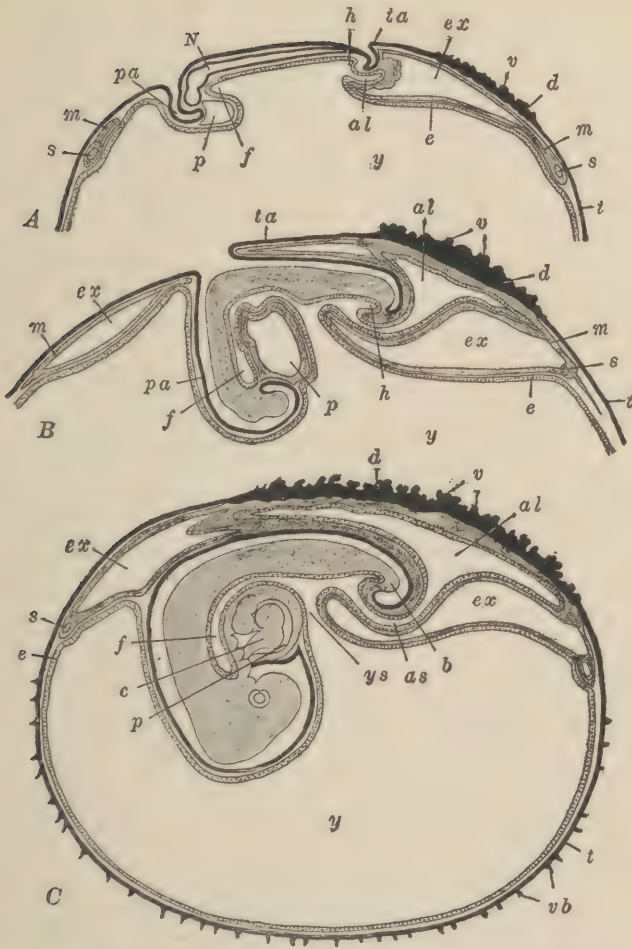
Fig. 227. — Diagrams of the formation of the embryonic membranes and appendages in the Rabbit. From Kellicott (Chordate Development). After Van Beneden and Julin (partly after Marshall). Sagittal sections. A. At the end of the ninth day, after coitus. B. Early the tenth day. C. At the end of the tenth day. Ectoderm black; endoderm dotted; mesoderm gray.

al. Allantois. as. Allantoic stalk. b. Tail bud. c. Heart. d. Allantoidean trophoderm (see page 490). e. Endoderm. ex. Exocœlom. f. Fore-gut. h. Hind-gut. m. Mesoderm. N. Central nervous system. p. Pericardial cavity. pa. Proamnion. s. Marginal sinus (sinus terminalis). t. Trophoblast. ta. tail-fold of amnion. v. Trophodermal villi. vb. Trophoblastic villi. y. Cavity of yolk-sac. ys. Yolk-stalk.

be noted that during or soon after the above processes, mesoderm has been proliferated between the ectoderm and the underlying endoderm in a manner to be described below. Moreover, there has arisen within this mesoderm the usual cœlomic split, separating it into the somatic and splanchnic layers. In either type (*a*) or (*b*), the amnion is then formed by folds of ectoderm and somatic mesoderm, which arise about the rim of the flattened embryonic knob (i.e., the blastodermal ectoderm), in essentially the same manner as in the Chick (Fig. 227). Thus as the amnion is completed by the meeting of the folds at the sero-amniotic connection, the chorion is at the same time reëstablished above it. This portion of reëstablished chorion now consists therefore, not
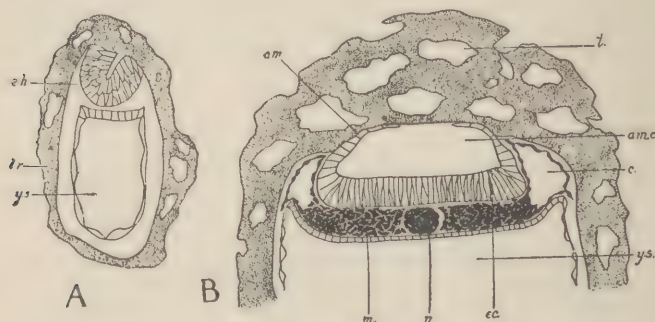
Fig. 228. — Formation of the amnion in the Hedgehog (*Erinaceus*). From Jenkinson (Vertebrate Embryology). After Hubrecht. A. Early. B. Later stage.

tr. Trophoblast. *y.s.* Yolk-sac. *e.k.* Embryonic knob. *am.* Amnion. *l.* Lacuna. *ec.* Ectoderm of embryonic plate. *n.* Notochord. *m.* Mesoderm. *c.* extra-embryonic cœlom.

only of an outer layer of ectoderm, but also of an inner layer of somatic mesoderm. Between the latter and the somatic mesoderm of the amnion is of course the extra-embryonic cœlom.

There are, however, certain minor points of difference to be noted between the case of the Bird and that of the placental Mammal. In the first place there is the origin of the chorionic ectoderm. In the Bird this arises entirely from ectoderm of the extra-embryonic blastoderm which has grown out over the yolk. In the Mammal, on the other hand, since the folds arise just at the border between blastodermal ectoderm (embryonic knob) and trophoblast, a large portion of the ectoderm in the folds; i.e., that of the outer layer, seems to be formed from the latter substance. Thus while the lining of the amnion may be partly blastodermal, the ectodermal part of the chorion which

covers it is apparently entirely of trophoblast, a tissue which seems to have no real homologue in the Bird. A second, but rather less important difference between Bird and Mammal is the fact that in the latter the tail fold often appears earlier than the head fold, and is therefore the longer of the two.

II. The Second Method of Amnion Formation. — In the second method of amnion formation, the trophoblast above the embryonic knob is never interrupted, a condition known as *entypy*. In contrast to method I, the amniotic cavity then arises merely as a space within the embryonic knob or in connection with the knob and the trophoblast above it. Here again, however, there are variations in the process, so that it may best be described under the headings, Type (*a*), Type (*b*), and Type (*c*).

Method II Type (*a*). — This type is illustrated by the Hedgehog (*Erinaceus,* Fig. 228) in which the rudimentary amniotic cavity appears, not in the knob itself, but as a space between the center of its dorsal side and the trophoblast. The edges of the knob, however, remain adherent to the trophoblast, and these edges now turn and grow toward one another between the trophoblast and the cavity. Thus when they meet and fuse, the ectodermal layer of the amnion is completed. Later, the extra-embryonic cœlom lined by mesoderm forces its way in between the trophoblast (chorionic ectoderm) and the ectoderm of the amnion, so that in this manner the latter receives its mesodermal covering and the former its mesodermal lining. It may be noted that the type of amnion formation thus exemplified by the Hedgehog is quite similar in many respects to that just described under method I, and may, therefore, represent a transitional stage between methods I and II. Later, as the embryo develops, the edges of the flat blastoderm are folded downward in the usual manner, and portions of the mesodermal layers are of course involved in this process. The layer lying next to the endoderm is then splanchnic mesoderm, and the one next to the ectoderm (either trophoblastic or embryonic) is somatic mesoderm.

Method II Type (*b*). — The second type of method II is typically illustrated in the development of the Guinea Pig (*Cavia*), in which the process is as follows:

Shortly after gastrulation is completed, the embryonic knob becomes separated from the trophoblast above it, and moves down near the opposite side of the blastocyst. In so doing, it pushes the central

portion of the endodermal layer before it; the edges of this central portion, nevertheless, remain attached to the dorsal trophoblast. This process presently results in the production of a clear space between the knob and the trophoblast, bounded on its sides by the upstretching endoderm. A cavity now develops in the middle of the embryonic knob; this is the rudiment of the amniotic cavity (Fig. 229, *A*, *B*). On the floor of this cavity, the cells remain columnar, and are homologous with the upper or ectodermal layer of the embryonic portion of the blastoderm in previous forms. The cells of the roof and sides, on
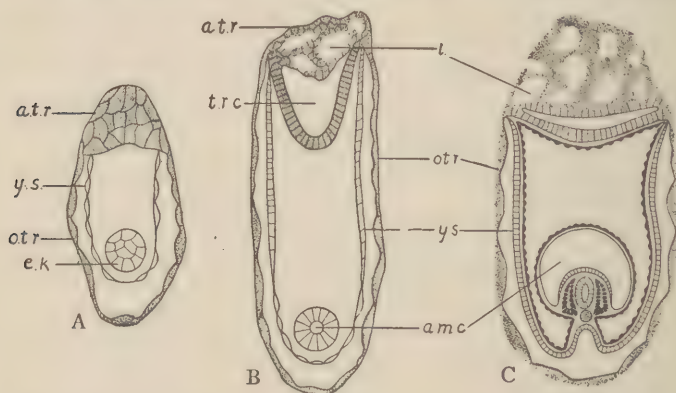
Fig. 229. — Formation of the amnion in the Guinea Pig (*Cavia*). From Jenkinson (Vertebrate Embryology). After Selenka. A. Early. B. Later. C. Latest stage.
a.tr. Allantoidean trophoderm. *o.tr.* Omphaloidean trophoblast (see page 488). *l.* Lacuna. *e.k.* embryonic knob. *am.c.* Amniotic cavity. *y.s.* Yolk-sac endoderm.

the other hand, soon flatten and form the ectodermal layer of the amnion. The latter now begins to expand, filling the space above it (Fig. 229, *C*). In the meantime mesoderm begins to arise between the ectoderm of the blastoderm and the endoderm beneath it, while within this mesoderm the cœlomic split occurs, producing two layers. These layers then spread out upon either side, the lower layer extending over the endoderm as the splanchnic mesoderm, and the upper layer extending up over the ectoderm of the amnion as the somatic mesoderm. The amnion is now completely formed, and consists, as in previous cases, of an outer layer of mesoderm and an inner one of ectoderm. Further development merely involves an increase in size and a gradual folding in about the embryo to form the umbilical stalk.

In anticipation of the method which is next to be described under

type (c), however, it may finally be added that besides the amniotic cavity thus formed, there has also arisen a cavity in the dorsal trophoblast from which the knob was separated. This second space is often referred to as the *false amniotic cavity*, but in the type under discussion it never has any connection with the true cavity. It presently disappears and has no further significance.

Method II Type (c). — This last type of amnion formation is well shown in the Mouse (*Mus*, Fig. 230). In this form the embryonic knob moves down as in the Guinea Pig, pushing the endoderm before it, but does not become separated from the trophoblast. Instead, the latter simply thickens, thus filling up the space which would otherwise result. A cavity now appears in the upper part of the knob, and at once comes into communication with a cavity in the lower part of the thickened trophoblast; i.e., the false amniotic cavity. The mesoderm next arises between the endoderm and the ectoderm of the knob, whence it spreads upward between the endoderm and the thickened trophoblast. Within this mesoderm the cœlomic split next develops upon either side, and the two cœlomic spaces then press towards each other and finally unite. In this manner the mass of ectoderm and trophoblast, including the cavity, is cut in two in approximately the region where the ectodermal and trophoblastic elements were in contact. This process is such as to leave one closed cavity in the trophoblast and another closed cavity in the embryonic knob, with the extra-embryonic cœlom lined by mesoderm between them. The cavity in the knob is, of course, the amniotic cavity with its usual layers, while the one in the trophoblast is the false cavity already referred to. The latter, it will be noted, is in no wise different from its homologue in type (b), except that in this case it temporarily communicates with the true cavity. Later, as in the former case, it disappears.

The Inversion of the Germ Layers. — Before passing on to a discussion of the relative primitiveness of methods I and II, it is worth while to note a peculiar misconception which arose in the minds of early students of forms like *Cavia* and *Mus*. These are cases, it will be recalled, where the embryonic knob moves far down into the blastocyst. The obvious result is that the endoderm extends well up on either side, considerably above the level of the blastoderm. Hence, if in examining the blastocyst of such a form, the investigator overlooked the outer layer of trophoblast, the first layer he would come to would be endoderm. He would thus get the impression that in
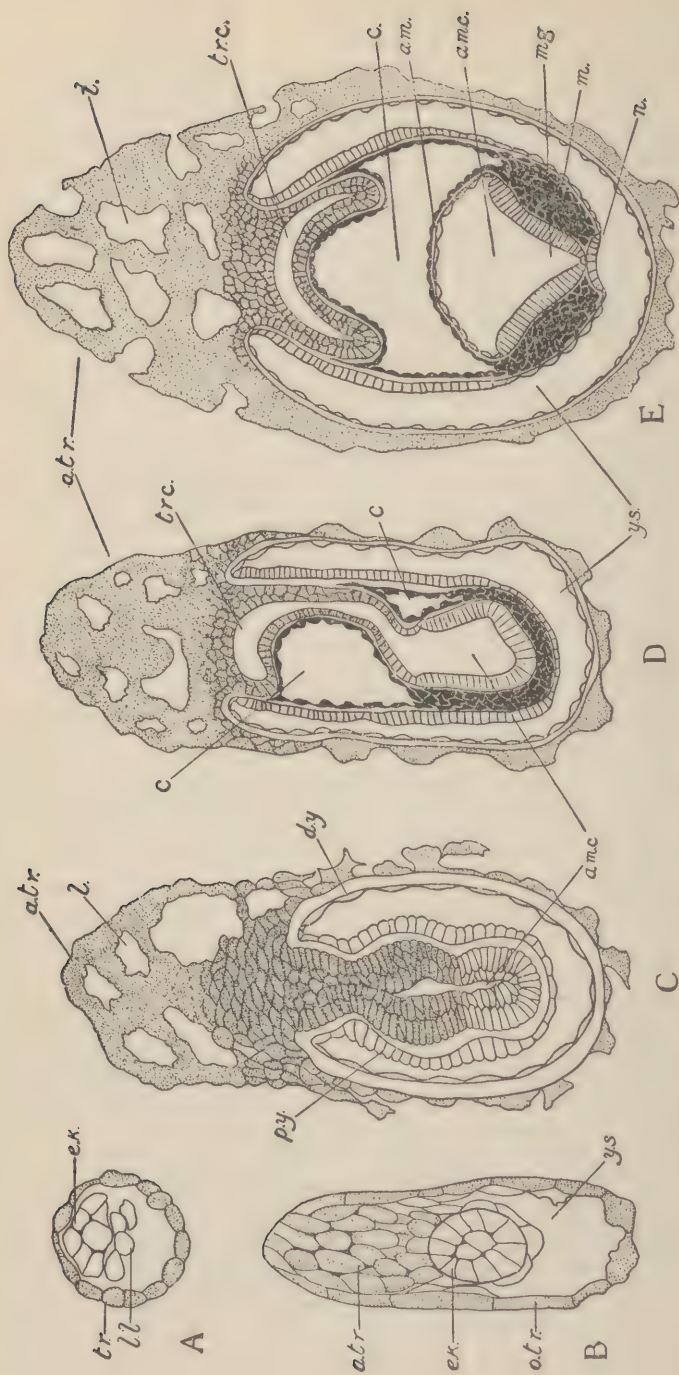
Fig. 230. — Formation of the amnion in the Mouse (*Mus*). From Jenkinson (Vertebrate Embryology). A-E. Successive stages. *tr.* Trophoblast. *a.tr.* Allantoidean trophoblast. *o.tr.* Omphaloidean trophoderm. *e.k* Embryonic knob. *l.l.* Lower layer, i.e., endoderm. *y.s.* Yolk-sac. *py.* Proximal or upper, and distal or lower walls of yolk-sac. *l.* Lacuna. *l.* Lacuna. *am.* Amnion. *am.c.* Amniotic cavity. *tr.c.* Temporary trophoblastic or false amniotic cavity. *c.* Extra-embryonic cœlom. *m.g.* Medullary groove. *m.* Mesoderm. *n.* Notochord.

some mysterious manner the endoderm had gotten on the outside of the blastocyst. This oversight was exactly what occurred, and the phenomenon was, therefore, referred to as an " inversion of the germ layers." As a matter of fact, it is now clear, that no such inversion really exists, and hence the phrase is of only historical interest.

The Relative Primitiveness of Methods I and II. — There has been some discussion as to which of these two main methods of amnion formation is the more primitive among placental Mammals, one view — that of Hubrecht — being strongly in favor of method II. The reasons for this attitude are based chiefly upon the characteristics of the mammalian chorion indicated in connection with method I, and are as follows: In the Bird or Reptile (i.e., the Sauropsids), there is, as suggested, no chorion (the layer corresponding in relative position to the mammalian trophoblast) until it is formed by the outer walls of the amniotic folds. In all the Mammals whose early development is known, on the other hand, the blastocyst is entirely enclosed in trophoblast, or chorionic ectoderm, before any amnion has been formed, either by folds or otherwise. It is true that in those cases where the process of folding occurs (e.g., in the Rabbit), the original trophoblastic chorion above the embryo virtually disappears, and the new one in this region is formed from the outer walls of the folds. Nevertheless, even in these cases there is no denying that there was a trophoblastic chorion previous to the folding, and further that most of the (chorionic) portion of the folds is still really trophoblastic. Hence, as indicated above, it is said that the original trophoblastic chorion of Mammals can not be regarded as homologous with the layer of the same name in the Sauropsids. From this statement it then follows, according to proponents of this idea, that the cases of the formation of the mammalian amnion and chorion by folds could not have been derived from this process in the Reptiles; it must rather represent a reversion to the reptilian condition, or else a piece of independent evolution.

There are, however, many zoölogists who do not subscribe to the theory just presented. Instead they regard method I as the more primitive, for the following reasons: In the first place it is known that Mammals as a class sprang from Reptiles, in which group the method of amnion formation is by folds as in the Birds. Furthermore, among those Mammals which are in other respects most primitive; i.e., the Monotremes and Marsupials, the formation of the amnion by folds

(according to the evidence of those stages which are known in these animals) in all probability prevails. Lastly, as admitted by the opponents of the view now being presented, the trophoblastic chorion of the Mammal is not really homologous with the true chorion of the Bird; it is rather a secondary development, whose early and complete enclosure of the blastocyst is made possible by the absence of yolk. Consequently, though the trophoblast usually takes a large part in the formation of the mammalian chorion, it has not, contrary to the argument stated in the foregoing paragraph, necessarily anything to do with the formation of the amnion. Indeed, as has been seen, the latter frequently forms by folds in spite of the presence of the precocious trophoblastic chorion, and those cases where it does not (method II) are merely another secondary development. In conclusion, it may be said that on the whole the arguments for the conception just presented appear to be rather more cogent and reasonable that those opposed to it.

The Primitive Streak and Related Structures

It will have been noted that during the process of amnion formation (in method I, slightly preceding it) there arises in one way or another from the embryonic knob a flat plate of ectoderm. This area of ectoderm together with the endoderm directly beneath it is the area from which the embryo proper is now to develop. As has been suggested, in the Chick it is termed the embryonic blastoderm; in the Mammal it is the *embryonic shield*.

The Primitive Streak and the Mesoderm. — The *primitive streak* arises along the mid line of the embryonic shield in what later proves to be the longitudinal axis of the embryo. As in the Bird, it results from a thickening of the ectoderm extending from the future posterior edge of the shield to a point somewhat anterior to the center. Here it terminates in a knot or node called *Hensen's knot,* as in the Chick. On either side of the primitive streak, cells are presently proliferated from it and extend out as a sheet between the ectoderm and endoderm; they constitute the *mesoderm* (Figs. 231 and 232).

The Notochord and the Primitive Groove and Pit. — At the same time a rod of tissue is differentiated in front of Hensen's knot, forming a typical *head process* which apparently gives rise to the *notochord*. Running along the middle of the streak, there is also often a *primitive groove,* which terminates in the region of the knot in a distinct *primitive*
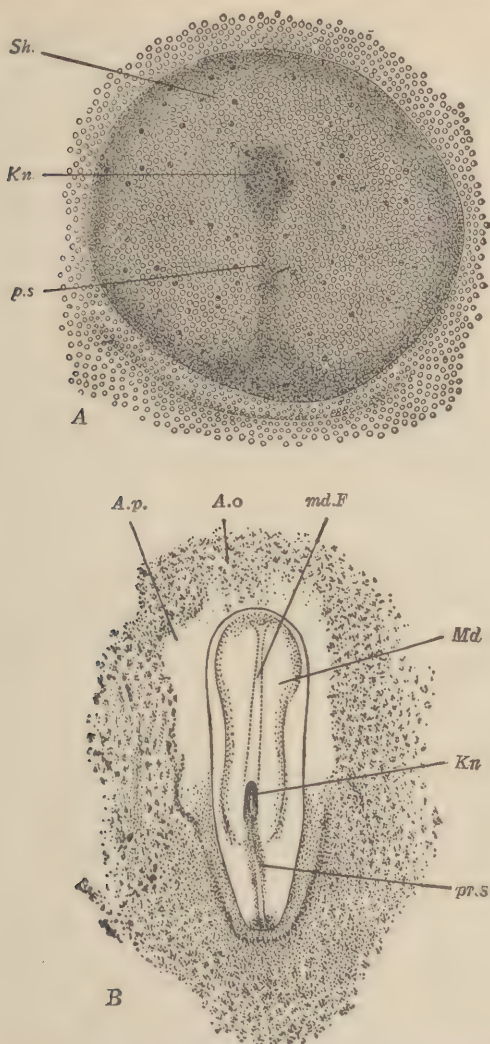
Fig. 231. — A. Surface view of embryonic shield of a Dog of thirteen to fifteen days.
B. Surface view of embryonic shield of Dog showing medullary plate, etc. From Minot
(Laboratory Text-Book of Embryology). A. After Bonnet.

A.o. Area opaca. *A.p.* Area pellucida. *kn.* Hensen's knot or node. *Md.* Medullary
plate. *md.F.* Medullary furrow (groove). *ps., pr.s.* Primitive streak. *Sh.* Embryonic
shield.

pit. Although the groove may sometimes be faint or absent, the pit on the other hand is frequently more strongly developed than is the case among most Birds. Thus, instead of being a mere depression, the pit may penetrate both ectoderm and endoderm and form an opening into the cavity of the yolk-sac or, as it may be termed, the *archenteron;* e.g., in Man. In other instances, however, instead of making a simple perforation, the pit may extend forward into the head process for a short distance as a canal. The canal may then finally penetrate into the archenteron or it may end blindly. When the pit thus enters
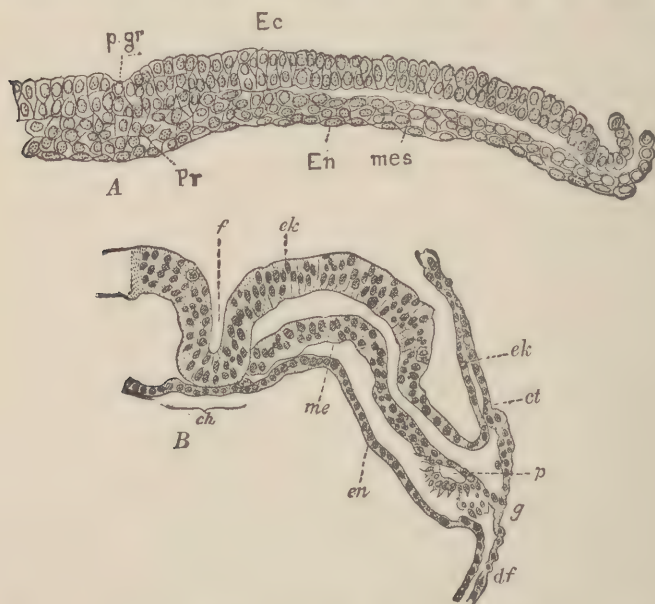
Fig. 232. — A. Transverse section through the primitive streak of the Mole. B. Transverse section through a Human embryo of 1.54 mm. (Graf von Spee's Embryo *Gle*). From Minot (Laboratory Text-Book of Embryology), after Heape (A), and Graf von Spee (B).

ch. Notochord. *ct.* Somatic mesoderm of amnion. *df.* Splanchnic mesoderm. *Ec.* or *ek.* Ectoderm. *en.* or *En.* Endoderm. *df.* Dorsal furrow. *g.* Junction of extra-embryonic somatic and splanchnic mesoderm. *me.* or *mes.* Mesoderm. *p.* Rudiment of embryonic cœlom. *p.gr.* Primitive groove. *Pr.* Primitive streak.

the head process, it is sometimes referred to as the *notochordal canal.* Nevertheless, either a direct perforation or a short canal in such a position is extremely suggestive of the anterior remnant of a closing blastopore. As such, in a form like the Frog, it would soon become

the neurenteric canal by virtue of its enclosure by the medullary folds. In the Mammal, it is perhaps doubtful whether the perforation, when present, ever persists until the fusion of the neural folds in this region is actually complete. During its existence, however, the opening in question is so clearly homologous with the *neurenteric canal* of the Frog that it is frequently so named (Fig. 233).
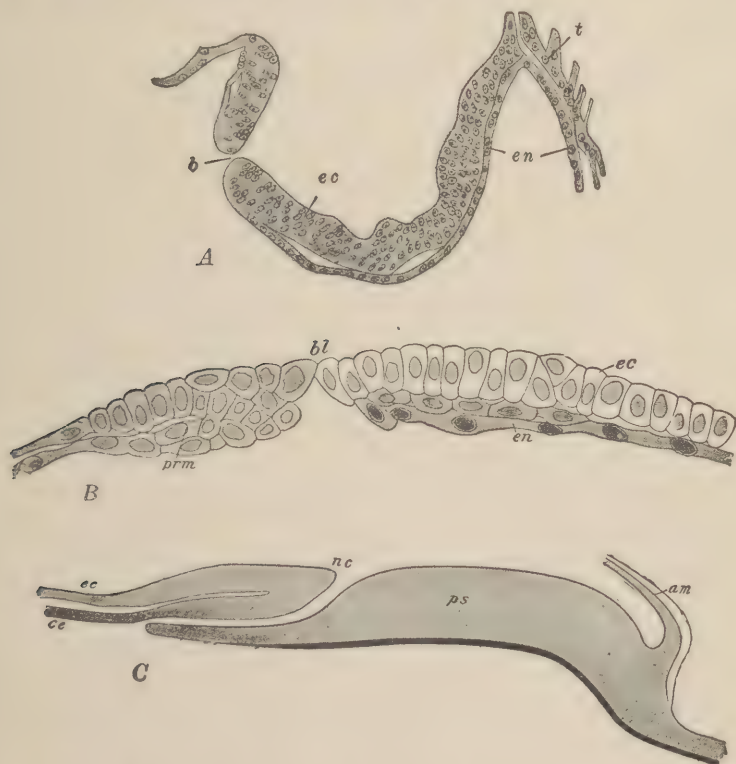
Fig. 233. — A. Sagittal section through the embryonic shield of the Hedgehog, showing the transitory blastopore. From Kellicott (Chordate Development). After Hubrecht. B. Posterior part of a sagittal section through the embryonic disc of the Mole. C. Diagram of a sagittal section through the embryonic disc of the Mole. From McMurrich. (Development of the Human Body). After Heape.

am. Amnion. *b.* or *bl.* Blastopore. *ce.* Chorda endoderm. *ec.* Ectoderm. *en.* Endoderm. *nc.* Neurenteric canal. *prm.* Peristomial mesoderm. *ps.* Primitive streak. *t.* Trophoderm.

The Nature of the Mammalian Primitive Streak. — From the above description it is very evident that the parts here indicated are

virtually homologous with the similarly named structures in the Bird. Not only is this true, but it may be added that very much the same arguments for and against the interpretation of the primitive streak of the Bird as the remains of an elongated *blastopore* may be applied to the case of the Mammal.

Thus, considering the negative side of the question first, it may be noted that in the Mammal as in the Bird there is no direct connection demonstrable between the blastopore and the streak. In fact in the placental Mammals, this point is rendered even more cogent by the following considerations: In the latter case, gastrulation is entirely by delamination, and a real blastopore scarcely exists at any time except insofar as the neurenteric or notochordal canal may be regarded as the remnant of one. Also in connection with this fact one of the strong affirmative arguments falls to the ground; i.e., since there is no genuine blastopore in these Mammals the demonstration of an apparent concrescence of the lips to form a primitive streak as in the Pigeon is impossible. Secondly, as in the Bird, the primitive groove, representing supposedly the remains of the actual blastoporal opening, never communicates with the archenteron. Finally, the primitive ridges forming the sides of the groove and representing the *blastoporal lips* have, as in the Bird, no connection with the endoderm beneath.

On the other hand, the arguments in favor of regarding the primitive streak of the Mammal as in some way the homologue of a blastopore are, save for the lack of proved concrescence, equally forceful. Thus, the differentiation of the embryo again occurs just in front of the primitive streak, and apparently the notochord arises in connection with its anterior end; i.e., its " dorsal lip." Also the mesoderm originates from the sides of the streak, and the *anus* forms at its posterior end. Lastly, not only does the primitive pit occur at the anterior end of the streak, but in the Mammal this pit is more marked than is usual in the Bird, and not infrequently it forms a virtual neurenteric canal.

Considering all the above facts, the following may now be stated in conclusion: The homology of the mammalian primitive streak with a blastopore is probably less well established than in the case of the Birds, and some embryologists are unwilling to admit it. Nevertheless, it is probably safe to say that even in the Mammal this structure is a vestige of at least part of what was once an actual blastopore.

THE YOLK–SAC, THE ALLANTOIS, AND THE
PLACENTA: THEIR STRUCTURE AND
FUNCTIONS IN THE MAMMAL

Among the Amniota of which the Chick is a type; i.e., the Birds, the chief organs through which the embryo receives its nutriment and effects respiration have been seen to be respectively the yolk-sac and the allantois. Among the vast majority of the Amniote group known as Mammals, however, these organs are very largely, and in many cases completely, supplanted in these functions by a new structure, typically associated with the *allantois* and termed the *placenta*. The large group of Mammals among whose members this organ is most fully developed is therefore known as that of the placental Mammals, a group which has already been frequently referred to. It will presently appear, however, that within this group there are certain types of placentas which vary from one another, both in their structure, and in the degree to which they have assumed the place and functions of the allantois and the yolk-sac. There exist also two relatively small mammalian groups, the *Monotremes* and the *Marsupials,* whose members possess either no placenta at all or only a very rudimentary one. Under these circumstances, therefore, it appears most convenient to treat the subject by taking up the conditions of the above organs in one group at a time. The Monotremes and the Marsupials will be considered first, since they are most primitive, and exhibit a condition most nearly akin to that in the Reptiles and Birds. After these there will be discussed certain orders of truly placental Mammals which best illustrate the various types of allantoic placenta, and perhaps suggest its method of evolution. The orders to be thus considered are the *Ungulates,* the *Carnivores,* the *Rodents,* and the *Primates.* Finally before passing to a study of the first group, it may be mentioned incidentally that the discussion of this subject also necessarily involves in each case a more extended reference to the matter of implantation referred to above.

THE MONOTREMES

These curious mammalian forms comprise the Spiny Ant Eater (*Echidna*), and the Duck Bill (*Ornithorhynchus*). They are remark-

able as Mammals in that they lay hard-shelled eggs like Birds. As might be expected in such a case, the yolk-sac is well developed and filled with yolk, while the allantois is also prominent. The placenta, on the other hand, because of its peculiar nature and functions, which its study will presently reveal, is naturally entirely lacking. In short, in eggs of this sort the embryonic parts under discussion are in all respects characteristically reptilian or avian (Fig. 234, *A*).

The Marsupials

This group comprises the Kangaroos (*Macropodidæ*), the Opossums (*Didelphyidæ*), the Marsupial Cats (*Dasyuridæ*) and the Bandicoots (*Peramelidæ*). These animals are all characterized by the fact that their young are born in a comparatively undeveloped condition. They then crawl inside of the Marsupial pouch of the mother and become attached to her teats, where they remain for some time. As might be expected under such circumstances the means for obtaining nourishment and aerating the blood previous to birth are very primitive. In fact, among the various members of the group there occur some very excellent examples of graded transition from the condition in the Monotremes to that in the real placental Mammals. The Opossum is perhaps as primitive a form as any in this respect, and will therefore be considered first.

The Most Rudimentary Type of Placenta. — In *Didelphys*, or the Opossum (Fig. 234, *B*), the yolk-sac, as in all the Marsupials, is well developed though it contains no yolk. Nevertheless, upon its upper surface there is a clearly defined area vasculosa, bounded by a sinus terminalis. Since there is no yolk, however, the nutriment which the above area is to convey into the embryo must be obtained from some other source; this is accomplished in the following manner: Although the mesoderm, and consequently the area vasculosa, do not reach to the opposite side of the yolk-sac, the endoderm on that side comes into contact with the trophoblast of the blastocyst. During implantation this trophoblast then becomes thrown into folds (not shown in the figure) which fit into depressions in the uterine wall; the latter secretes a viscid fluid known as *uterine milk*. This is then absorbed via the trophoblast and endoderm, and finally reaches the embryo, partly at least by the way of the area vasculosa. This contact of the embryonic trophoblast and the uterine tissue may be regarded as a very

primitive beginning of what will later be recognized as a placenta. The allantois is very small in this case, as in most other Marsupials, and has no contact with the trophoblast. The exact means by which the embryonic blood is aerated, therefore, is a little uncertain. Very possibly, however, it also is accomplished through the contact of yolk-sac and maternal tissues.
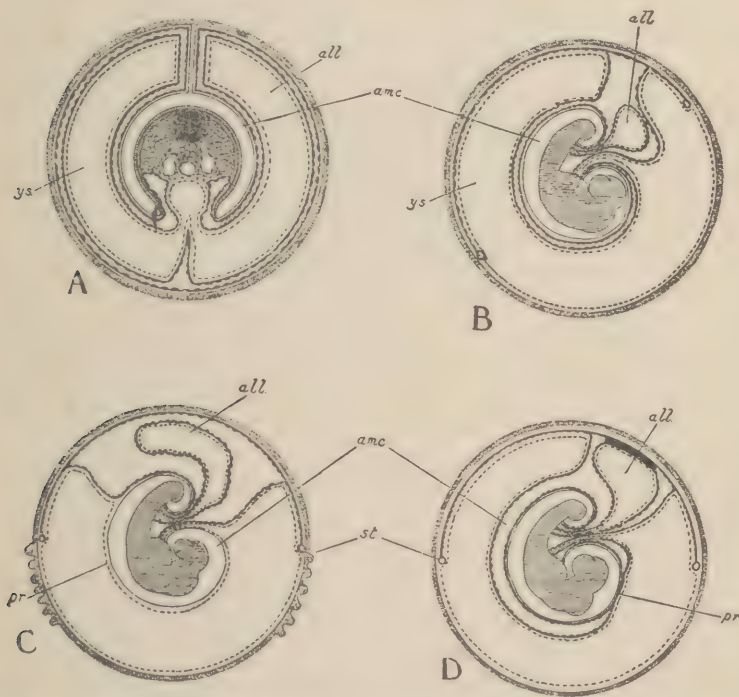
Fig. 234. — Fœtal membranes of A, Monotremata; B, C, D. Marsupials. B. *Phalangista, Aepyprymnus, Didelphys, Bettongia*; C. *Dasyurus*; D. *Perameles* and *Halmaturus*. (In *Didelphys* the proamnion persists as in *Dasyurus*.) From Jenkinson (Vertebrate Embryology). (A, B, D, after Semon; C, after Hill).

In this diagram of Mammalian fœtal membranes the trophoblast (ectoderm of mammalian chorion) is stippled, the ectoderm of the amnion represented by a continuous line, the endoderm by a broken line, and the mesoderm (somatopleure and splanchnopleure) by a thick line swollen at intervals.

all. Allantois. *am.c.* Amniotic cavity. *pr.* Proamnion; ie., portion of amnion without mesoderm. *y.s.* Yolk-sac. *s.t.* Sinus terminalis of area vasculosa.

A "Yolk-Sac Placenta." — *Dasyurus* is the second form to be considered, because it exemplifies the next step in the development of a true placenta (Fig. 234, C). The allantois, however, is still small, and

the placenta-like structure which occurs is, therefore, again associated entirely with the yolk-sac. Furthermore, the trophoblast in contact with the non-vascular area of the sac once more forms the connection with the uterine wall. In this instance, however, this implantation is more thoroughgoing, and there appears for the first time that process of uterine erosion so noteworthy among some of the higher forms. This erosion is accomplished by the trophoblast which, after becoming thickened and syncytial (i.e., trophodermal) in certain regions, eats into the uterine epithelium and engulfs some of the maternal blood vessels. The blood so obtained passes in between the trophoblast and yolk-sac, and is absorbed. Presumably also such an arrangement makes respiration possible in the typically mammalian manner; i.e., by exchange of gases between embryonic and maternal blood. The type of contact which is here illustrated is so intimate that the area in which it occurs is sometimes referred to as a *yolk-sac placenta*.

A Primitive " Allantoic Placenta." — Finally, the most advanced condition in this Marsupial series is illustrated in *Perameles,* where the following situation occurs (Fig 234, *D*): Here the yolk-sac is again large, and possesses an area vasculosa which is probably functional in absorbing some nourishment by way of the trophoblast. In this case, however, the allantois also is well developed, and comes into contact with the trophoblast. Implantation then occurs and the trophoblast in the area of this contact becomes attached to the uterine wall, whose epithelium in this region is transformed into a vascular syncytium. The trophoblast finally disappears, and the maternal blood vessels come into intimate contact with those which have grown out through the mesoderm of the allantois (Fig 235). Thus there is established a true *allantoic placenta*. As will presently appear, however, the exact relationship of its embryonic and its maternal parts is different from that described in any of the subsequent types.

In connection with this, the first real placenta to be noted, there is one very important fact to be pointed out. Neither in this placenta nor in those of any other type does the fœtal and the maternal blood actually mix. It is always completely separated by one or more membranes. Through these membranes, however, it is easily possible for an exchange of nutritive and waste materials, as well as gases, to take place.

This completes the account of the Marsupials, and we are now prepared to pass on to the orders of the genuine placental Mammals. As

has been indicated, the latter are so named because here an allantoic placenta of one sort or another becomes the usual and chief means of embryonic nutrition and respiration. In the Marsupials, on the other hand, such a condition occurs only in the single instance last cited.
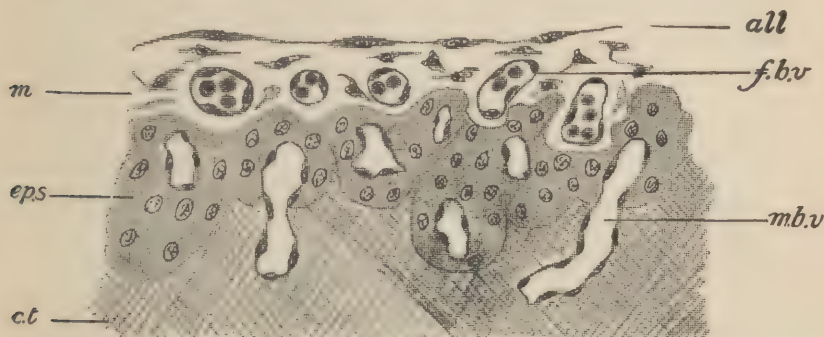
Fig. 235. — Section through the placenta of *Perameles*. From Jenkinson (Vertebrate Embryology). After Hill.

all. Allantoic epithelium. *m*. Mesoderm of allantois together with mesoderm of chorion. *f.b.v*. Fœtal blood-vessel. *ep.s*. Syncytium of uterine epithelium. *m.b.v*. Maternal blood-vessels. *c.t*. Sub-epithelial connective tissue of uterus.

THE PLACENTALIA OR TRUE PLACENTAL MAMMALS

Within this large group, the embryonic appendages whose condition is being considered are probably in their most primitive form among the Ungulates, and this order, therefore, will be treated first.

The Ungulates.

The Early Means of Nutrition and the Yolk-Sac. — Before the blastocyst enters the uterus, the latter has been prepared for its reception by the period of the procestrum. As a result of this preparation, the uterus contains a mixture of epithelial cells, hæmatids and leucocytes, together with fat, proteid, and glycogen, secreted by the uterine glands. This substance, again termed uterine milk, is eagerly absorbed by the trophoblast of the blastocyst. Meanwhile, the yolk-sac is developing and soon attains a relatively large size. It also possesses an area vasculosa, and it is very likely that in these early stages it functions actively in helping to absorb and to pass on the nutriment obtained for it through the trophoblast. Later, however, the yolk-sac becomes insignificant, its function being entirely taken over by the allantois and the placenta, whose development will now be described.

The Placenta and the Allantois. — The blastocyst of this group, it will be remembered, soon becomes greatly elongated. The trophoblast of this elongated vesicle is then produced either into ridges (Pig) or finger-shaped processes; in any case they contain a core of chorionic mesoderm and are called *villi*. These may completely cover the vesicle (Horse and Pig); they may be arranged in bunches or *cotyledons* (Cow and other Ruminants); or they may be in cotyledons with individual villi scattered over the intercotyledonary areas (Giraffe). According to the arrangement thus indicated, the placenta which develops will be known respectively as *diffuse, cotyledonary,* or *inter-*
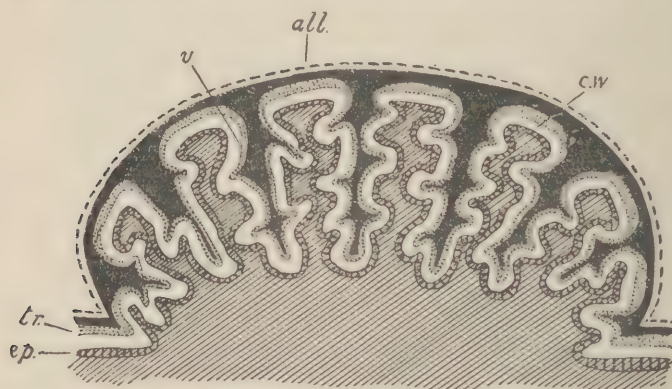
Fig. 236. — Diagram of a fœtal and maternal cotyledon of the Cow. From Jenkinson (Vertebrate Embryology).

all. Allantoic epithelium. *tr.* Trophoblast. *v.* Villus. *ep.* Uterine epithelium continued into crypt. *c.w.* Wall of crypt. The maternal connective tissue is shaded.

mediate. During the process of implantation of the blastocyst which is now going on, the developing villi are fitted into various crypts, which appear at this time over the surface of the uterus. These also are necessarily arranged diffusely or in groups according to the arrangement of the villi. Indeed, in the case of cotyledonary placentas, the locations where most of the groups of crypts (*maternal cotyledons*) appear during pregnancy are permanent, existing as raised areas (*caruncles*) even in the uterus of an unborn calf. Thus, in these instances the locations of the embryonic cotyledons are secondary, being determined by the positions of the maternal caruncles (Fig. 236).

Meanwhile, by the time the villi are well in place, the allantois has grown out and reached the mesodermal lining of the chorion. In the

Ungulates it then grows rapidly, and soon far outstrips the yolk-sac, becoming in fact much larger than the amnion (Fig. 237). The mesoderm which covers it carries the umbilical blood vessels and this mesoderm together with the capillaries of the blood vessels mingles with the chorionic mesoderm in the core of each villus. In this manner, the fœtal vessels are brought near to those of the mother in the tissues
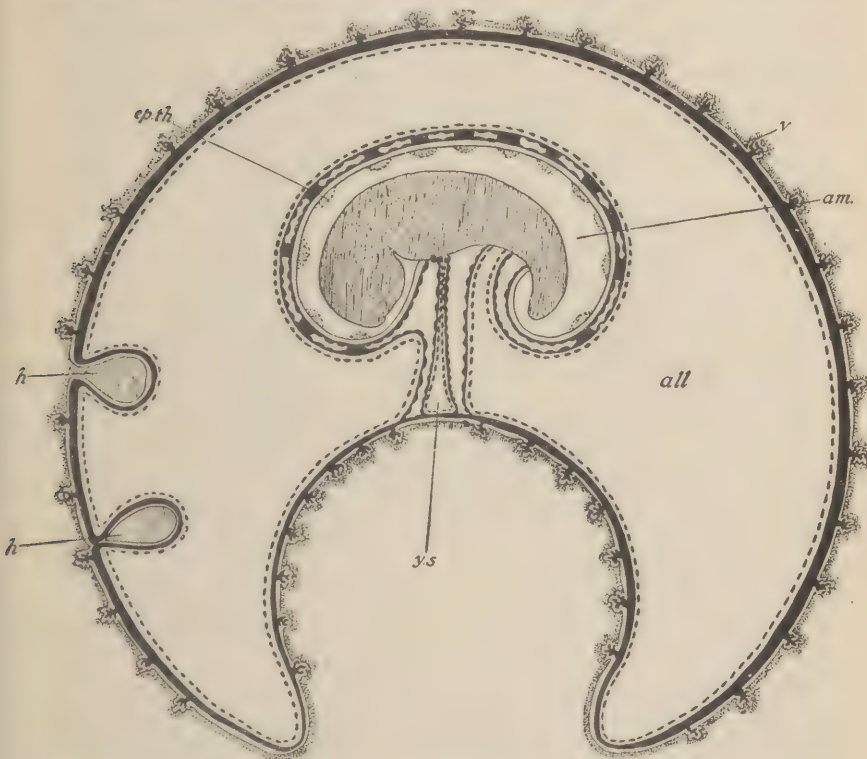
Fig. 237.—Fœtal membranes of the Horse, later stage. From Jenkinson (Vertebrate Embryology). After Bonnet.

all. Allantoic cavity. *am*. Amniotic cavity. *ep.th*. Epithelial thickenings of amnion. *h*. Hippomanes, small bodies formed in the allantois by the inpushing of accumulations of uterine milk. Later these break loose and float about, finally becoming hardened by saturation with calcium oxalate. *v*. Villus. *y.s*. Yolk-sac.

of the crypts. When this has occurred, the placenta may be said to be complete.

It is to be especially noted that in the processes just described there is absolutely no erosion of the uterine epithelium, which was

restored following the prœstrum.[6]   Each villus simply fits into a
crypt, like a plug in a socket, from which it is easily withdrawn at the
time of birth.   Indeed, during the period of gestation, the maternal
epithelium continues to secret nutritive substances between the crypt
walls and those of the villi.   This is absorbed by the trophoblast and
taken up by the embryonic vessels, so that in this case, as in some
others, the embryonic nutriment is not all obtained directly from that
which is carried in the maternal blood.   A placenta in which the con-
tact between fœtal and maternal tissue is such as indicated is often
defined as *indeciduate*.   This term implies that at the time of parturi-
tion, the wall of the uterus is literally not deciduous.   That is, there
is no tearing away of maternal tissue when the fœtal part of the
placenta separates from that of the mother.

The Carnivores.

The Yolk-Sac. — As in the Ungulates, the period of the prœstrum
results in the accumulation within the uterus of a nutritive mixture
somewhat similar to that already described.   In some cases, however
(e.g., the Cat), it appears to be less abundant than in the Ungulates,
and of a more watery consistency.   The uterine epithelium is then
reëstablished, and everything is ready for the reception of the blasto-
cyst.   In this case, also, the latter begins its development by absorption
of the uterine milk.   A yolk-sac has meanwhile developed, and ap-
parently it plays about the same part in this process as was noted
in the Ungulates.   As in that order, also, this appendage later be-
comes relatively insignificant (Fig. 238).

The Placenta and the Allantois. — While these events are occurring,
a change is taking place in the uterine wall.   In a band which com-
pletely encircles this wall the epithelium disappears.   Likewise, in the
region of a corresponding band about the equator of the somewhat
elliptical blastocyst, the latter begins to adhere to the prepared uterine
wall.   During this process of implantation, trophoblastic villi similar
to those of the Ungulates begin to develop from the wall of the blasto-
cyst in the region of its adherence.   Because of the obvious band or
zone-like shape of this region, the type of placenta which develops
in this order is called *zonary*.   The villi of the trophoblast, which
may contain a core of mesoderm, now push their way directly into
the mucous tissue of the uterus.   As they do so, they absorb any re-

[6] According to some authorities there is erosion of the maternal epithelium
in the Ruminants.

maining epithelial debris which comes in their way. In this manner, they soon become firmly embedded in the maternal tissue and surrounded by maternal blood vessels. While this is going on, the allantois has grown out, and as in the Ungulates, soon becomes the chief append-
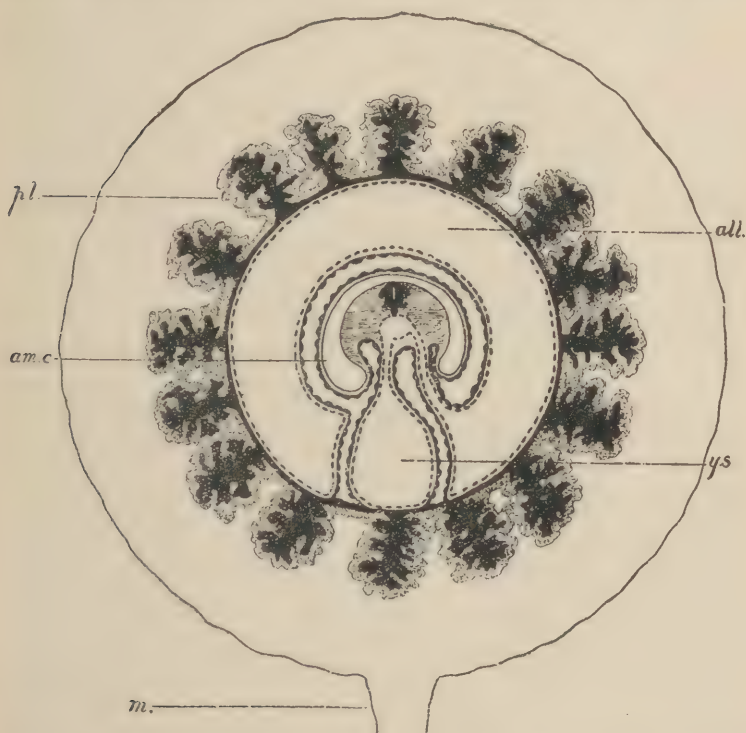
Fig. 238. — Fœtal membranes and placenta of the Dog. from Jenkinson (Vertebrate Embryology). After Duval.

all. Allantois. *am.c.* Amniotic cavity. *m.* Mesometrium, or sheet of connective tissue attaching the uterus to the body wall. *pl.* Zonary placenta. (See text under description of the placenta of the Carnivores for the definition of this term.) *y.s.* Yolk-sac. The fœtal mesoderm, connective tissue and blood vessels are in black.

age of the embryo. When the allantoic mesoderm comes into contact with the chorionic mesoderm in the zone of implantation, the allantoic capillaries penetrate the villi, and the placenta is virtually complete. During subsequent development, however, it becomes thickened somewhat by growth and branching of the villi and capillaries, and also of the maternal connective tissue in which they are embedded. The

glands of the latter continue to supply debris and fat, which is absorbed by the trophoblastic villi up to the end of gestation. The main source of embryonic nutrition, however, is presumably material contained in the maternal blood (Fig. 239).

It will be noted that the attachment of the fœtal and the maternal parts of the placenta is much more intimate in this case than it was
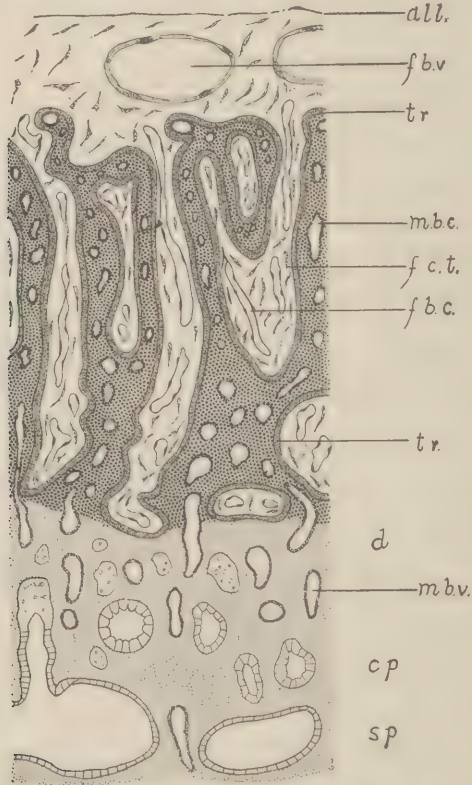
Fig. 239. — Section through the placenta and uterine wall of the Cat. From Jenkinson (Vertebrate Embryology).

all. Epithelium of allantois. *f.b.v*. Large fœtal blood-vessels. *f.b.c*. Fœtal capillaries. *f.c.t*. Fœtal connective tissue. *tr*. Trophoblast (finely shaded). *m.b.c*. Maternal blood capillaries; these are immediately surrounded by maternal connective tissue (coarsely stippled). *m.b.v*. Maternal blood-vessels passing through the layer of glandular detritus (*d*) *cp*. Compacta (necks of glands). *sp*. Spongiosa (dilations of glands).

in the Ungulates. This has resulted from the disappearance of the uterine epithelium, which allows the capillaries in the fœtal villi to

come that much nearer to those of the mother. Because of this very close attachment, it also happens that at birth a large portion of the maternal tissue is torn away with the fœtal portion of the placenta. For this reason, this type of placenta may be regarded as *deciduate*. Indeed, as will appear from a study of the remaining groups, the Carnivores are probably the only animals possessing a placenta of which this is true in any large degree.

The Rodents. — As in the forms previously studied, the uterine epithelium is reconstructed following the prœstrum, and is, therefore, present when the blastocyst reaches the uterus. The method of attachment and of placenta formation which now follows varies somewhat in different Rodents, although it is fundamentally similar in all of them, and leads to practically the same results. It will further be found that in this case, the former process; i.e., attachment or implantation, is somewhat elaborate, and therefore requires more detailed attention than has hitherto been necessary. The chief conditions with respect to this process as well as to the general character of the yolk-sac, may be illustrated by reference to two forms, the Mouse and the Rabbit.

Implantation and the Development of the Yolk-sac. — In the case of the Mouse, the elongated uterus becomes lined with pits upon its *anti-mesometric* side. This is the side opposite its point of attachment to the cœlomic wall, the latter region being termed the *mesometric* side. Each of the blastocysts, of which there are several in the Mouse, becomes embedded in one of these pits with the embryonic knob facing the narrow lumen of the uterus (Fig. 240, *B*). The further history of one of them will suffice. As soon as the embedding has occurred, the trophoblast immediately starts to erode the epithelium of the pit, and to devour the debris which results. Meantime the blastocyst enlarges sufficiently so that the side containing the embryonic knob crosses the uterine lumen and comes in contact with the opposite wall (Fig. 240, *B, C*). In this way, each blastocyst obtains attachment at every point, and completely obliterates the cavity of the uterus where it is situated. At every place where contact is thus established; i.e., on the bottom and sides of the original pit, and also upon the uterine wall opposite to it, erosion of the uterine epithelium is carried on. The placenta, which will presently be described, is established on the mesometric side of the uterus at the second point of contact, and therefore next to the embryo. Then, owing to the intimate relation of trophoblast and allantois in this region, the trophoblast (trophoderm) on this

side of the blastocyst is called *allantoidean*. On the opposite side; i.e., at the original bottom of the pit, the uterine lumen is later again established. Here for a while epithelium once more develops, and covers both the wall of the uterus and the blastocyst (Fig. 240, *D*). Inside the latter, the yolk-sac has meanwhile formed, and on its upper surface has acquired an area vasculosa. Its lower wall, on the other hand, which is in contact with the trophoblast of the blastocyst, finally
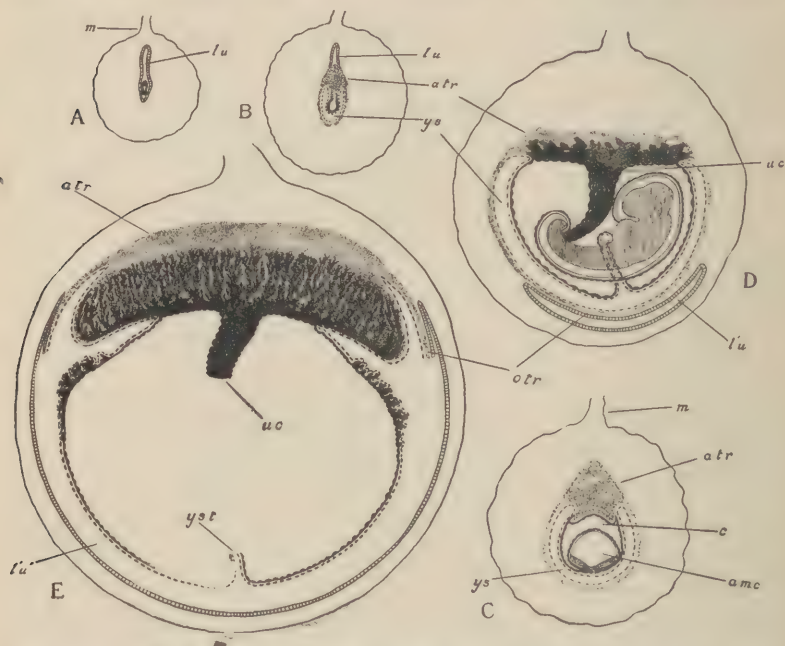
Fig. 240. — Five stages in the formation of the placenta in the Mouse. From Jenkin-son (Vertebrate Embryology). A. The blastocyst free in the uterus. B. The blastocyst attached and the placental thickening of the allantoidean trophoblast (trophoderm) developed (*a.tr*). C. Later stage, after closure of the amniotic cavity (*am.c*) and the obliteration of the uterine lumen. D. Placenta becoming established, and reappearance of uterine lumen (*l'u.*). E. Elaboration of the placenta. Disappearance of the distal wall of the yolk-sac and omphaloidean trophoblast (*o.tr.*).

c. Extra-embryonic cœlom. *l'u'*. New uterine lumen on the anti-mesometric side. *lu.* Original lumen of the uterus. *y.s.* Yolk-sac. *y.st.* Yolk-stalk. *u.c.* Umbilical cord. *m.* Mesometrium.

degenerates. The trophoblast (in this region termed *omphaloidean*) and the newly formed epithelium at this point then also vanish, and thus the interior of the yolk-sac is placed in immediate communication with the reëstablished uterine cavity (Fig. 240, *E*).

Turning now to the method of implantation in the Rabbit, it is found to be somewhat less complicated. Here a pair of folds arise upon the mesometric side of the uterus, and the blastocysts become attached to these. Each blastocyst in this case lies between the folds and becomes attached by the trophoblast on either side of the embryonic
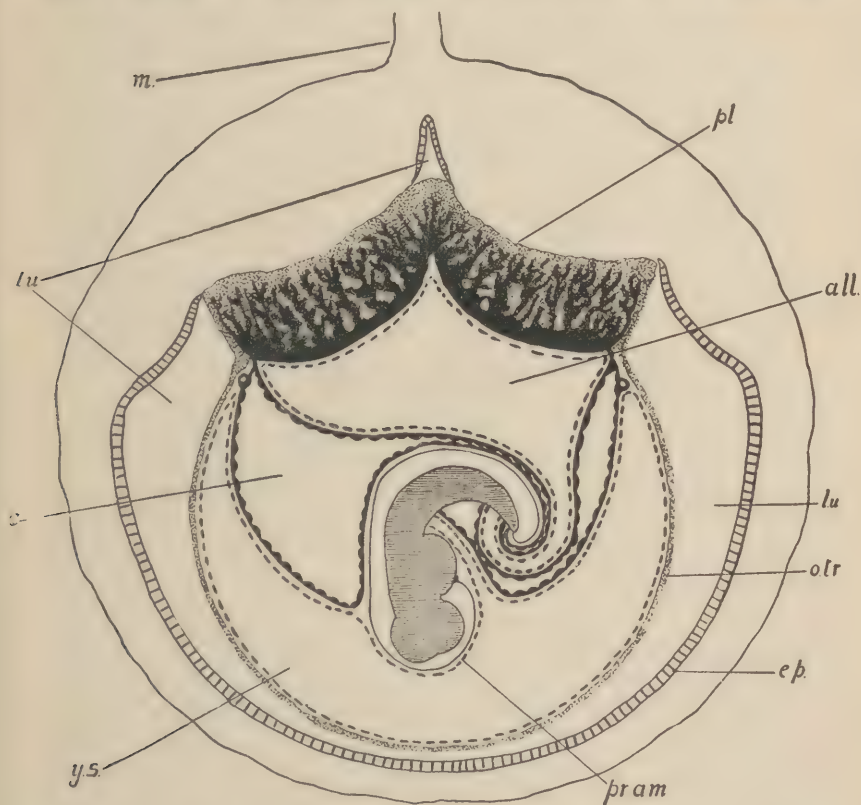
Fig. 241.   Fœtal membranes and placenta of the Rabbit. From Jenkinson (Vertebrate Embryology).   After Duval and Van Beneden.
    pr.am. Proamnion.   Other letters as in Fig. 240.

shield.   In these regions, the uterine epithelium is eroded, and two placentas are established which later merge into one (Fig. 241).   The opposite side of the blastocyst forms no intimate contact with the uterine wall and presently disappears.   Concurrently the ventral wall of the yolk-sac also disappears, so that again, as in the case of the

Mouse, the cavity of the sac is directly continuous with that of the uterus (this stage not shown in the figure).

Having thus described the two chief types of implantation among the Rodents, we are now in a position to discuss the nature of the placenta and other means of nutrition common to all this group.

The Placenta and the Allantois. — During the erosion of the uterine epithelium indicated above, the allantoidean or placental trophoblast becomes greatly thickened, trophoblast of this character being called *trophoderm.* This thickened trophoblast then continues to eat down into the mucous layer of the uterine wall, engulfing, as it does so, maternal blood vessels, together with glycogen from the glycogen-filled cells (*maternal glycogen tissue*). There next appear in the trophoderm numerous *lacunæ,* and into these is emptied the maternal blood from the vessels whose walls have been destroyed (Fig. 242, *A*). Meantime an allantois has arisen. In the Rodents, the endothelial portion of this organ containing the cavity is usually small, although in the Rabbit, which in this as in most other respects is more primitive, the allantoic cavity attains a considerable size (Fig. 241). The mesodermal part, however, is always well developed, and soon reaches the trophoblast or trophoderm of the placental region, bringing with it the umbilical blood vessels (Fig. 242, *B*). The capillaries of these vessels then penetrate the trophoderm so as to come near to the cavities containing the extravasated maternal blood. This blood is being constantly poured into the central space of the placental region, and withdrawn at the periphery through the maternal veins. Gradually toward the maternal side, the trophoderm surrounding the lacunæ becomes further vacuolated through the secretion of glycogen, thus establishing a *trophodermal glycogen tissue* (Fig. 242, *C*). Eventually, through the increase of the latter, the layer of original maternal glycogen tissue is entirely eliminated.[7] Such is the character of the completed placenta of the Rodents, which, because of its development upon only one side of the blastocyst, has the general shape of a disc or button. It is, therefore, termed *discoidal,* as distinguished from the zonary form found in the Carnivores.

Comparing the placenta in this case with that noted in the Carnivores, the chief difference will be found to be that, in the completed organ of the Rodents, maternal tissue plays very little part. The

[7] The maternal glycogen tissue is said to be more abundant and persistent in the Rabbit.

placenta indeed is principally composed of the fœtal trophoderm with its capillaries, lacunæ, and glycogen tissue. This difference seems to be achieved by the fact that the trophoderm erodes not only the uterine
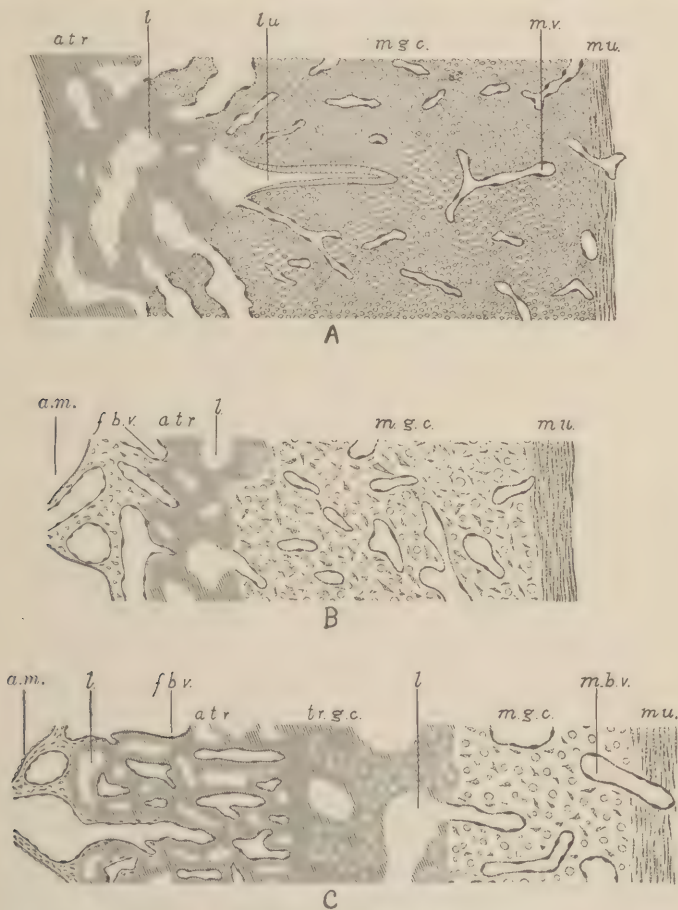
Fig. 242. — Placentation of the Mouse. Details of the five stages of Fig. 240. From Jenkinson (Vertebrate Embryology).

A. Strip of a section through the allantoidean trophoblast (trophoderm) and overlying maternal tissues in stage C, Fig. 240.

atr. or *a.l.* Allantoidean trophoderm. *mu.* Muscularis. *m.v.* Maternal blood-vessel, opening below into *l.* lacunae of the trophoderm. *l.u.* Original lumen of the uterus. *m.g.c.* Maternal glycogen tissue.

B. Similar strip of the same parts in stage D, Fig. 240.

f.b.v. Fœtal blood-vessel. *a.m.* Allantoic mesoderm. Other letters as in A.

C. Similar strip of the last stage, Fig. 240.

tr.g.c. Trophodermal glycogen tissue. Other letters as in B.

epithelium, but a large part of the mucosa and its blood vessels as well. Because of this peculiar structure, it happens at parturition that, aside from the blood in the lacunæ, very little real maternal tissue is lost. This follows from the fact that the actual line of separation runs through the region of vacuolated cells which have now lost their glycogen and collapsed, and this region, as noted, is held to be entirely
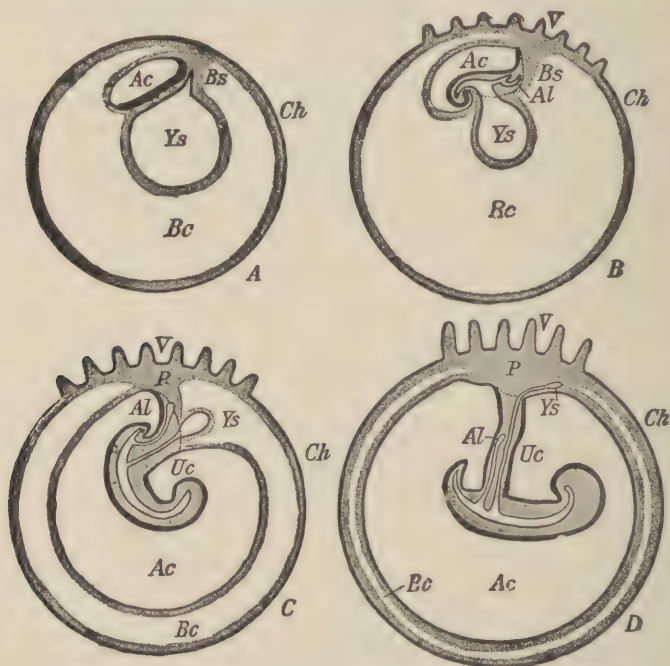
Fig. 243. — Diagrams illustrating the formation of the umbilical cord and the relations of the allantois and yolk-sac in the Human embryo. From McMurrich (Development of the Human Body). The heavy black line represents the embryonic ectoderm; the dotted line marks the line of the transition of the body (embryonic) ectoderm into that of the amnion. Stippled areas, mesoderm.
    Ac. Amniotic cavity. Al. Allantois. Bc. Exocœlom. Bs. Body-stalk. Ch. Chorion. P. Placenta. Uc. Umbilical cord. V. Chorionic (trophodermal) villi. Ys. Yolk-sac.

trophodermal. On account of this lack of maternal tissue to be torn away, many authorities regard the term deciduate as a misnomer when applied to placentas of this type. If the above description be correct, it apparently is a misnomer. Nevertheless, such placentas are still commonly classified under this head.

As regards the method of nutrition in this order, it is apparent that,

aside from the glycogen, nutriment is chiefly obtained, so far as the placenta is concerned, from the maternal blood. It will be remembered, however, that among the Rodents, the yolk-sac is always eventually open to the uterine cavity. Thus, for instance in the Mouse and the Rabbit, the lower epithelial wall of this organ was found to disappear completely, while in the Guinea Pig it is never even formed. This being the case, the upper wall of the sac can and does function actively in the absorption of uterine secretions throughout gestation. Hence both in its form and in its continued activity, the yolk-sac of the Rodent differs markedly from that of the types previously studied within the strictly placental group.

The Primates.[8]

The Allantois and the Yolk-Sac. — In the order of Primates, the nature of the yolk-sac and allantois is somewhat unique, while the latter organ is also peculiar in its method of development. An account of these structures will be given, therefore, before proceeding to the matter of implantation and placenta formation within this group.

First, as regards the allantois, it will be found that the endothelial sac is even more limited than it was in the majority of the Rodents. Furthermore, the mesoderm of that organ does not comprise, as in most previous cases, a mere covering for the sac; instead, it forms a thick stalk, the *body-stalk,* or *umbilical cord,* which attaches the embryo to the chorion or wall of the blastocyst. Into the proximal end of the mesodermal cord, the hollow endothelial element then projects for only a short distance (Figs. 243 and 244). This condition is brought about as follows: In the first place, it will be recalled that this group is one in which the endoderm of the small yolk sac either very early loses, or else never attains, contact with the trophoblast; instead, the larger portion of the original blastocœlic space is presumably occupied very early by a solid mass of mesoderm (Fig. 244, *A*). Then when the split in this mesoderm develops, it gradually extends all about the amnion and yolk-sac between these

[8] The characteristics of the embryonic appendages which are ascribed to this order apply to only one of the family of *Lemurs;* i.e., *Tarsius.* This animal, in respect to these organs, may be classed with the lower Monkeys. So far as is known, however, all other Lemurs are similar to the Ungulates as regards the yolk-sac and allantois, and also even in the possession of a diffuse indeciduate placenta. This exception must be borne in mind with reference to all statements concerning the Primates as a whole.
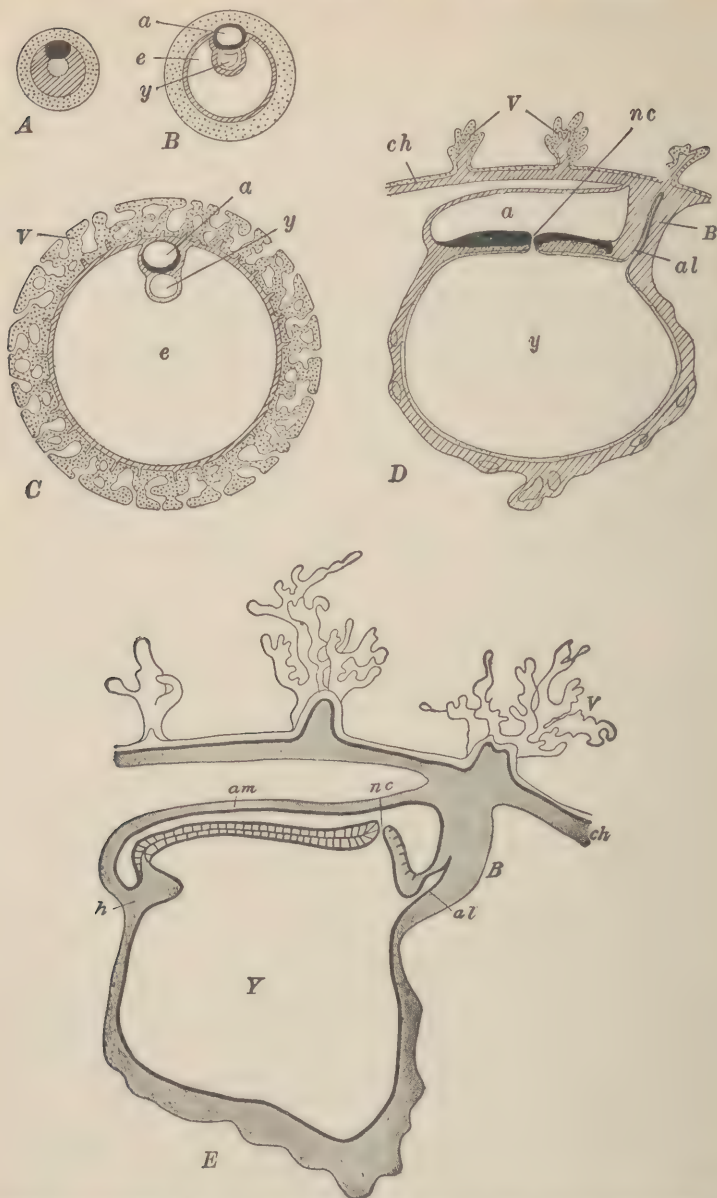
Fig. 244. — Diagrams of sagittal sections through the Human blastodermic vesicle, showing the formation of the amnion and trophoderm. From Kellicott (Chordate Development). A-D, after Keibel and Elze. E. From McMurrich (Development of the Human Body), after Graf von Spee. In all the figures the anterior end is toward the left, and in all the figures except E the following conventions are used: Black, embryonic ectoderm; heavy stipples, trophoblast and trophoderm; light stipples, endoderm. Oblique ruling, mesoderm. A. Hypothetical early stage; mesoderm a solid mass. B. Amniotic cavity and wide exocœlom established; endoderm limited to a small vesicle beneath the embryonic ectoderm. The exocœlom in reality contains scattered mesen-chyme cells. C. Blastodermic vesicle enlarged and covered with trophodermal villi, into which the mesoderm is extending. Endodermic vesicle (yolk-sac) very small (stage of Peter's ovum) D. Embryonic portion only, of an older vesicle showing the neurenteric canal, primitive streak (in the plane of the section posterior to canal), and body-stalk. The mesoderm of the yolk-sac is becoming vascular. E. Sagittal section through a Human embryo of 1.54 mm. (Graf von Spee's embryo Gle).
a. Amniotic cavity. al. Allantois. am. Amnion. B. Body-stalk (umbilical cord). ch. Cho-rion. e. Exocœlom. h. Heart region. nc. Neurenteric canal. V. Chorionic villi. Y. Yolk-sac.

structures and the wall of the blastocyst, with the exception of one
point at the postero-dorsal end of the future embryo. At this point the
split stops, thus leaving the above mesodermal connection or stalk.
Although at first located in this position, the embryonic end of the
stalk soon moves around so as to be attached to the embryo on its
ventral side. It retains, however, its original point of attachment to
the trophoblast, since it is here that the placenta is to be formed.[9]
From this description it is evident that in the Primates, the allantois, or
more strictly in this case, the umbilical cord, does not grow out from
the embryo to the trophoblast. It is there from the first.

As concerns the yolk-sac, it is only necessary to state that it is
very rudimentary, having little or no function. The space which
might otherwise be occupied by these appendages, however, is eventually
filled in this order by a very large amnion.[10]

Implantation and Placenta Formation. — It has already been stated
that menstruation in this group probably corresponds to the procestrum
among the lower forms. At any rate, following menstruation the
uterine epithelium becomes reconstructed, and presumably at about
this time, the blastocyst finds its way into the uterus. Implantation
then occurs, and again as in the case of the Rodents, the details of
this process vary somewhat. In this instance, the chief variation
occurs, so far as is known, between two groups; i.e., Tarsius, together
with the other lower Monkeys, and the higher Apes, together with Man.

As regards the first group; i.e., that of Tarsius and the Monkeys,
the description may be brief. The region of implantation may occur
on the dorsal or ventral wall of the uterus, depending upon the form
in question, and is not marked by either pits or folds, as in the Rodents.
Nevertheless, before fixation takes place and while the uterine epi-
thelium is still intact, there occurs here a more or less definite hyper-
trophy of the mucous layer. The trophoblast of the blastocyst soon
comes in contact with this area, and promptly erodes the epithelium.
A discoidal placenta then develops at that point, which is very similar,
if not identical, with that described for the Rodent. Later, a second

[9] In Tarsius the placenta is formed on the opposite side of the blastocyst,
and the stalk shifts its point of attachment to the trophoblast accordingly.

[10] Though not certainly known, it appears that the amnion in the Primates
(excepting the Lemurs, in this instance including Tarsius) is formed in a manner
similar to that described under method II; i.e., by the development of a cavity
in the embryonic knob. The process in this group differs from that described
under the second method, however in that in this case the embryonic knob does
not move down to the opposite side of the blastocyst.

similarly shaped placenta may form where the blastocyst comes in contact with the opposite side of the uterus. The umbilical cord, of course, reaches only one of these, but the two are connected by blood vessels (Fig. 245, only one placenta in this case).

Considering now the second group; i.e., the higher Apes and Man, it unfortunately happens that as regards the earliest stages very little is definitely known. This is chiefly due to scarcity of material, two of
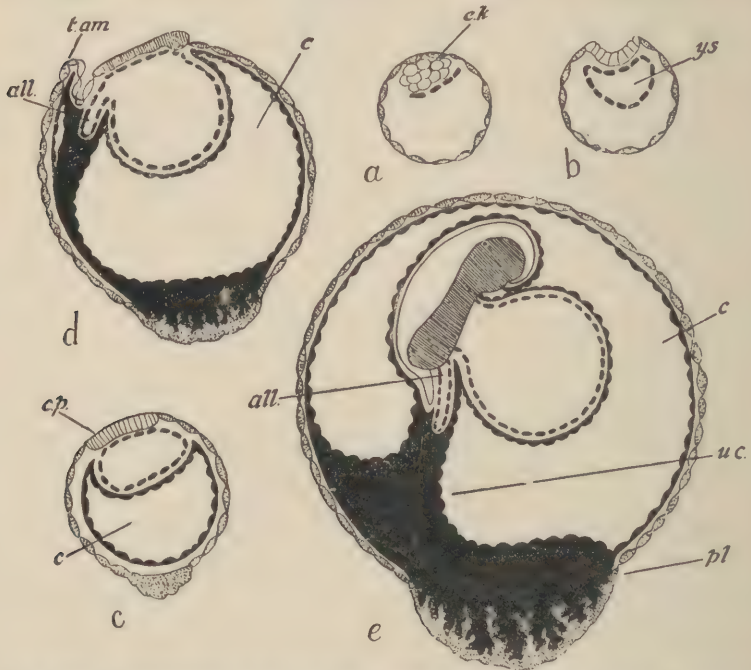
Fig. 245. — Development of the fœtal membranes in *Tarsius*. From Jenkinson (Vertebrate Embryology). After Hubrecht.

a. Blastocyst before Rauber's cells have disappeared. *b.* The embryonic knob, (*e.k.*) is being folded out to the surface; the yolk-sac is complete. *c.* The embryonic plate (*c.p.*) is at the surface, the extra-embryonic cœlom (*c*) is formed. *d.* The tail-fold of the amnion is growing forward (*t.am.*), the allantois (*all.*) has penetrated the mesoderm of the body-stalk, a placental thickening has been developed at the anti-embryonic pole. *e.* The amnion is closed and the body-stalk or umbilical cord (*u.c.*) is shifting its position, to be attached to the placenta (*pl.*).

the main sources of information concerning Man being the Bryce ovum and the Peters ovum. From the conditions which later obtain, however, fixation and development both in Man and the higher Apes is thought to be as follows:

The blastocyst usually becomes attached to the dorsal (i.e., posterior) wall of the uterus in Man, and to the ventral (i.e., anterior) wall in the Apes; here the trophoblast promptly starts its work of erosion. In this case, however, the process goes much further than in the instances so far noted. In fact, it is thought that by this means the blastocyst becomes completely buried in the mucous layer of the uterus, while the epithelium closes behind it. It thus virtually occupies the position of an internal parasite within the uterine tissue (Fig. 246). As growth now proceeds, the blastocyst, covered by a layer of uterine mucosa and some epithelium, begins to project into the cavity of the uterus. Meanwhile, it appears that practically the same changes are
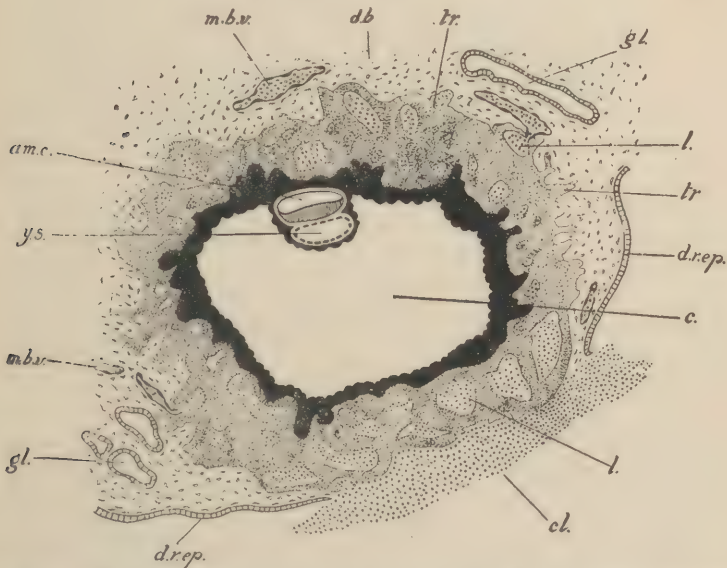
Fig. 246. — Early Human embryo with its membranes. From Jenkinson (Vertebrate Embryology). After Peters.

am.c. Amniotic cavity. c. Extra-embryonic cœlom. d.b. Decidua basalis (serotina). d.r.ep. Uterine epithelium covering the decidua reflexa or capsularis. l. Lacuna in trophoblast (tr.). gl. Uterine gland. m.b.v. Maternal blood-vessels opening here and there into lacunæ. cl. Clot marking (probably) the point of entrance of the blastocyst; here the uterine epithelium is interrupted. y.s. Yolk-sac.

taking place in the trophoblast, or chorion, as it may be called, as those which occurred in the Rodent; i.e., a thickening, and the formation of lacunæ. In this case, these processes by which the trophoblast is thus converted into the trophoderm at first occur on every side of the
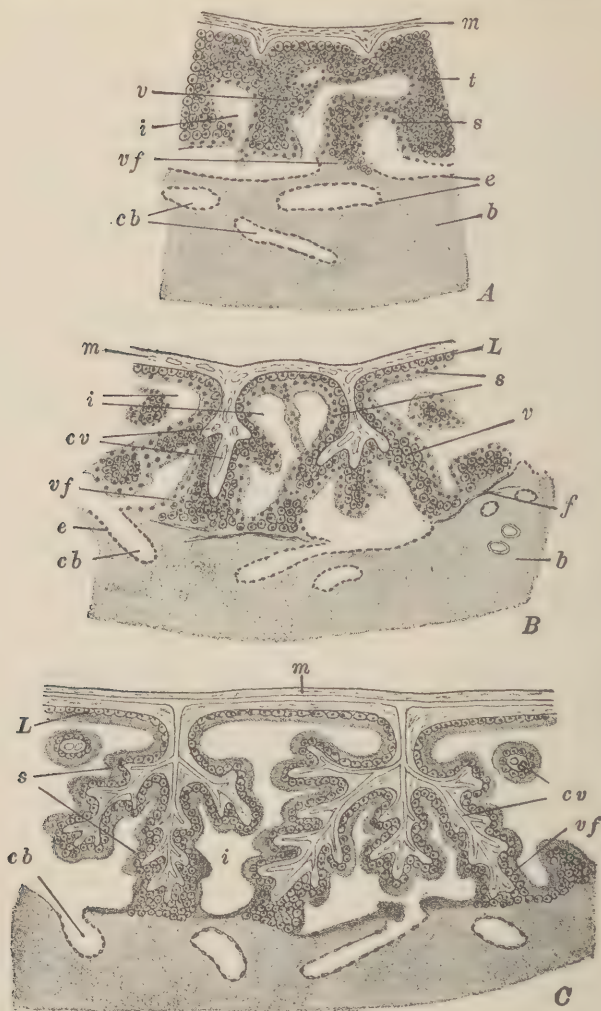
Fig. 247. — Diagrams illustrating the development of the "villi" in the Human placenta. From Kellicott (Chordate Development). A.B. After Peters. C. After Bryce. A. Chorionic mesoderm just beginning to extend into the villi. B. Mesoderm invading the villi which are now branched. Layer of Langhans cells forming beneath the syncytiotrophoderm. C. Continued branching of the villi, all now covered only by the syncytiotrophoderm and the single layer of Langhans cells.

b. Decidua basalis. cb. Capillaries of the decidua basalis. cv. Capillaries of the villi. e. Endothelium of the maternal capillaries. f. Fibrin deposited at the junction of the trophoderm and decidua basalis. i. Intervillous cavity (i.e., lacuna or sinus) filled with maternal blood. L. Langhans cells. m. Chorionic mesoderm. s. Syncytiotrophoderm. t. Trophoderm. v. Villi. vf. Fixation villi; i.e., those which extend clear across a sinus.
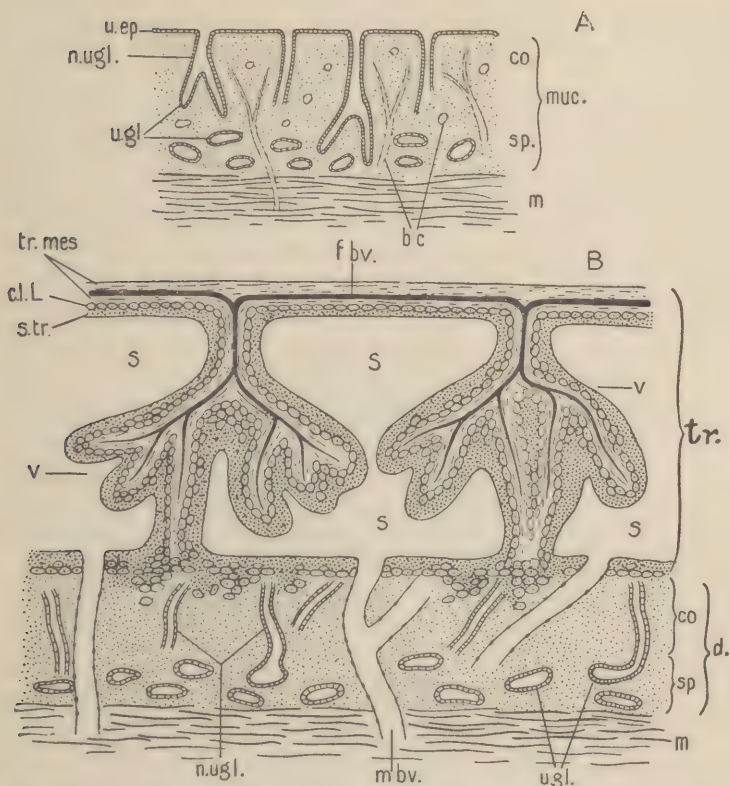
Fig. 248. — A. A diagram of an idealized section through the inner portion of the wall of the non-pregnant uterus a short time previous to the beginning of menstruation. The muscular layer is very thick, and only a small portion of it is shown. Beyond this layer on the outside of the uterus would come the peritoneal covering or serous membrane which here as elsewhere is quite thin. B. A diagram of a similar section through the Human placenta at a slightly later stage than that shown in figure 247 (according to Jenkinson). The trophoderm, it will be noted, has penetrated slightly into the compacta in this stage, so that the "villi" are more firmly attached. Note that these "villi" are quite different in their relation to the maternal tissue from that observed in the Ungulates, (Compare Fig. 236). No attempt has been made to distinguish between afferent and efferent blood vessels, although it is to be understood that both types exist on both the embryonic and maternal sides.

bc. Blood capillaries in the mucosa. c.l.L. Cell layer of Langhans, still clearly in evidence. co. Compacta. d. Decidua; for explanation of terms see further in text; fbv. Fœtal blood vessels. m. Muscular layer of uterus, or muscularis, only a small portion of which is shown. mbv. Maternal blood vessels. n.ugl. Necks of uterine glands in the compacta. s. Sinus lined by syncytial trophoderm, and filled with maternal blood. That the syncytial layer and cells of Langhans line the sinuses on the side of the decidua is questioned by some authors. sp. Spongiosa. str. Syncytial trophoderm. tr. Trophodermal region. tr.mes. Trophodermal (chorionic) mesoderm. u.ep. Uterine epithelium. u.gl. Uterine glands. v. "Villus."

blastocyst. Presently, however, the trophodermal development becomes much more marked on the inner side; i.e., that side away from the cavity of the uterus, and it is here that the permanent discoidal placenta is soon formed.

Throughout the trophoblast or chorion (now trophoderm) but especially on the placental side, the embryonic blood vessels, surrounded by a sheet of connective tissue (chorionic mesoderm), are working their way among the lacunæ, into some of which they project. These vessels and their connective tissue are covered with a thin trophodermal cell layer known in human embryology as the *cell layer of Langhans*. Outside of this, there is an added layer of the trophoderm
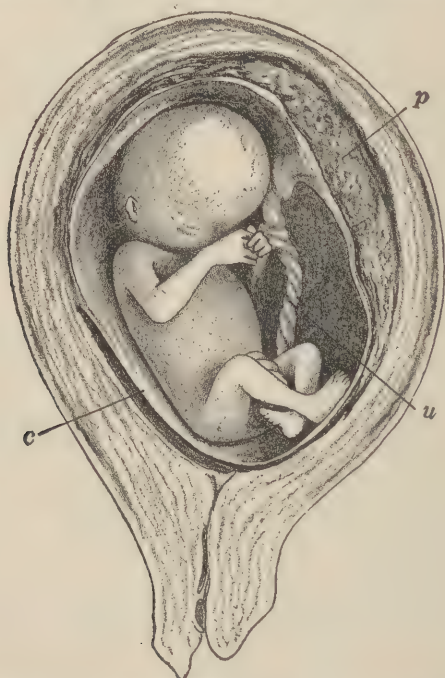
Fig. 249. — Human embryo of the fourth month *in utero*, showing the arrangement of the membranes and placenta. From Kellicott (Chordate Development).   After Strahl.
    c. Chorion and amnion. *p.* Placenta. *u.* Umbilical cord.

which is syncytial, and is apparently derived from the cells of Langhans, the latter being gradually used up. Thus, where the blood vessels, pushing their trophodermal and mesodermal layers before

them, project into the lacunæ, they have something like the appearance of villi, and are often so referred to (Fig. 247). It should be clearly understood, however, that these " villi " are in no sense homologous with the true villi described in connection with the indeciduate placenta of the Ungulates. They are not indeed essentially different from the capillaries which push into, and are hence covered by, the trophodermal material in the Mouse or Rabbit. As regards the lacunæ, they are again filled with maternal blood, and are often termed "sinuses." They also are lined by a syncytial layer of the trophoderm augmented to some extent by a layer of the cells of Langhans, similar to, and continuous with, that which covers the connective tissue of the fœtal capillaries (Jenkinson).

Outside of the discoidal placental region, the whole blastocyst is growing out so as to fill the cavity of the uterus (Figs. 249 and 250). Its wall in this area consists internally of extra-embryonic mesoderm. Next comes the trophoderm, in which in this region the " villi " and lacunæ are only slightly developed, and lastly comes the uterine mucosa and epithelium which covered the blastocyst after its embedding in the uterine wall. As growth continues, this epithelium is eventually bound to come in contact with that which lines the walls of the uterus at other points. By the time this occurs, however, the uterine epithelium and mucosa covering the growing blastocyst has become distended and is disappearing. Thus the trophoderm or chorion of this region is brought into direct relations with the epithelium which elsewhere still remains on the walls of the uterus, and this epithelium too presently disappears. Concurrent with the complete filling of the uterus and the disappearance of all its epithelium the chorionic layer of the blastocyst is everywhere united to the sub-epithelial mucosa of the uterine wall. It is only in the region of the placenta, however, that the chorion (trophoderm) normally continues to be vascularized and to thicken by the growth of villi.

The placenta, as so far described, consists then essentially of a greatly thickened layer of trophoderm containing lacunæ or sinuses filled with maternal blood, while into and across these sinuses extend trophodermal processes or " villi " containing fœtal connective tissue and capillaries. The layer thus indicated is obviously essentially tissue of embryonic origin, and is sometimes known as the " placenta proper." Between it and the muscular wall of the uterus there still exists a certain amount of the uterine mucosa; i.e., that part of the

mucosa which the trophoderm has not destroyed. It now remains to state that in some of the higher Apes and Man (as well as in certain of the lower animals already discussed; e.g., the Cat) this portion of the mucosa is itself differentiated into two main layers. The outer-
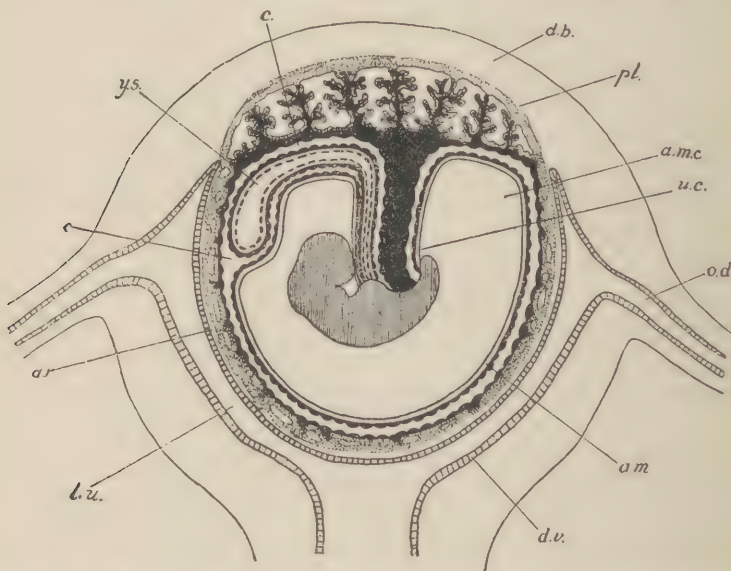
Fig. 250 — Diagrammatic section through the pregnant Human uterus and embryo at the seventh or eighth week. From Jenkinson (Vertebrate Embryology). After Balfour, after Longet.

am. Amnion. *a.m.c.* Amniotic cavity. The latter has enlarged until it occupies nearly all of the extra-embryonic cœlom (c), the amnion being reflected over the umbilical cord (*u.c.*) and yolk-sac (*y.s.*). The yolk-sac, it will be noted, is very small. *d.b.* Decidua basalis (serotina), in connection with which the trophoderm or chorion, represented everywhere by fine stippling, gives rise to the placenta. Thus the chorion in this region is the chorion frondosum. *d.r.* Decidua capsularis (reflexa), consisting of a thin layer of uterine epithelium and mucosa. It soon disappears, exposing the vacuolated trophoderm (chorion) beneath, which in this region becomes the chorion læve. *d.v.* Decidua vera, whose epithelium also disappears when the trophoderm beneath the capsularis (chorion laeve) comes in contact with it. *l.u.* Lumen of uterus, presently obliterated. *o.d.* Oviduct whose direction in the non-pregnant uterus would be nearly horizontal. *p.l.* Placenta; for details see Fig. 248.

most of these layers adjacent to the muscularis is filled with glands, and is known as the *spongiosa*. The second layer, to which the trophoderm is firmly adherent, and in which it is in fact slightly embedded, is occupied by the straighter smaller portions of these glands; i.e., their necks, and is called the *compacta* (Fig. 248). Moreover,

the compacta and spongiosa not only exist in the region of the placenta, but likewise at all other points around the uterine wall.[11] Thus, when the non-placental trophoderm of the enlarging blastocyst eventually comes into contact with this wall from which the epithelium has disappeared as indicated in the preceding paragraph, it becomes here also adherent to the compacta. During the later stages of pregnancy, both the compacta and spongiosa tend to degenerate and to become stretched and thin. It is then through the region of either one or both of these layers that the tissue breaks at the time of parturition.

This completes the description of the placenta and the adjacent regions in Man and the Apes. It remains, however, to indicate the names by which the various parts are known in human embryology. To understand the significance of this nomenclature, the student must bear in mind the older idea that placentas of this type were truly deciduate. That is, it was thought that a large part of the uterine wall was deciduous; i.e., torn away or shed at parturition. Hence those layers of the wall (i.e, the mucosa) which were supposed so to behave were termed the *decidua*. Also in correlation with this idea, most of the placenta and the covering of the blastocyst was supposed to be formed out of this decidua, rather than out of trophoderm. With this in mind, the reasons for the following names are fairly evident:

That part of the uterine wall to which the placenta is attached is known as the *decidua serotina,* or *decidua basalis* (Fig. 250). The portion of uterine mucosa and epithelium which, during the earlier development, covers the blastocyst on the side opposite the placenta, is called the *decidua reflexa* or *decidua capsularis*. That is, this portion, is, as it were, reflected over the blastocyst, forming a cover or capsule for it. Lastly, the remaining part of the uterine wall with which the decidua reflexa finally comes in contact is known as the *decidua vera*. As noted, when this contact occurs, the decidua reflexa disappears, and the decidua vera does so down to the compacta. Not only are the parts of the uterus thus distinguished, but the parts of the chorion are also defined. That part which forms the placenta and adheres to the decidua serotina is termed the *chorion frondosum*. The remainder, at least after its loss of the first slightly developed " villi," is the *chorion læve*.

[11] The spongiosa and compacta indeed occur not only in the pregnant Primate uterus, but in the non-pregnant uterus as well, particularly just previous to menstruation.

Comparing the means of embryonic nourishment in the Primates with those in the Rodents, there appears at least one notable difference. In the Rodents the yolk-sac plays a rather important part in obtaining nutriment for the embryo throughout development; in the Primates, on the other hand, this function, as well as that of respiration, is almost entirely subserved by the placenta. Coming to the actual structure of this organ itself, there exists a striking similarity between the two orders. There is also, however, a slight difference here, which is perhaps worth noting. At the time of parturition in the Rodents scarcely any maternal tissue, save blood, is lost, and hence the placenta is not at all deciduate in the strict sense of the word. In the Primates, on the other hand, there is a certain amount of the compacta and perhaps of the spongiosa lost at birth, and this is maternal tissue. Hence the Primate placenta, at least to this slight extent, may be said to be truly deciduate. The body-stalk in the two groups is in general similar in lacking any extensive endothelial element. As has been noted, however, its method of formation is different.
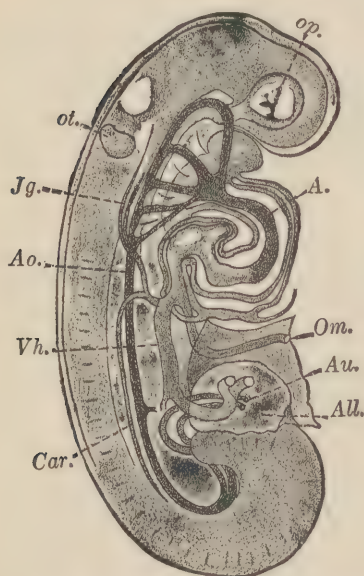
Fig. 251.—Reconstruction of a human embryo of 2.6 mm. From Minot (Laboratory Text-Book of Embryology.) After His.

A. Aortic limb of heart. *All.* Body-stalk. *Ao.* Dorsal aorta. *Au.* Umbilical arteries. *Car.* Posterior cardinal vein. *Jg.* Anterior cardinal vein (jugular vein). *Om.* Omphalomesenteric vein. *op.* Optic vesicle. *ot.* Otocyst. *Vh.* Right umbilical vein.

SUBSEQUENT DEVEL–
OPMENT

Aside from the description of the embryonic membranes and appendages, it has not seemed desirable to give a history of development in the Mammal subsequent to gastrulation and mesoderm formation. It may be stated, however, that in all fundamental respects, the general plan of growth and formation of the organs is virtually the same as that which has been described in the Chick. Thus the *medullary*

folds arise in the ectoderm anterior to the primitive streak, and beneath them the *somites* later develop within the mesoderm. From this it is evident that the parts of the future Mammal are to have the same relations to the structures already described as was the case with the Bird. The body with its primary layers whose origins have been indicated arises in the region of the blastodisc, or embryonic shield, by the processes of growth, differentiation, and folding off, already familiar. Finally it may be noted that the Mammal like the Chick passes through a stage where *visceral arches* and *pouches* are present, the latter being indicated externally by corresponding depressions or *furrows* (Figs. 251 and 252). Among Mammals it is true that in most cases these furrows do not quite connect with the pouches, so that actual
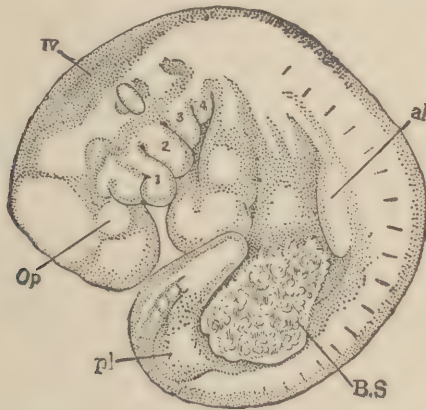
Fig. 252. — Human embryo of about twenty-three days (4.0 mm.). From Minot (Laboratory Text-Book of Embryology). After His (Embryo *a*).

al. Fore-limb bud. *BS*. Body-stalk. *Op*. Optic vesicle. *pl*. Hind-limb bud. *IV*. Fourth ventricle of brain. *1*. Mandibular process. *2*. Hyoid arch. *3, 4*. Third and fourth visceral arches.

slits opening into the pharynx are not established. Even within this group, however, genuine *visceral clefts* are occasionally found. Thus it is said that in the embryo of the common Cow (*Bos taurus*), the second pair of pouches regularly develop complete slits for a brief period (Anderson), while such openings have long been known to occur abnormally in other mammalian embryos; e.g., in that of Man. It is of course clear that in both Mammal and Bird the temporary presence of such functionless slits either actual or incipient is, like the occurrence of other vestigial structures, highly significant from an evolutionary viewpoint.

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